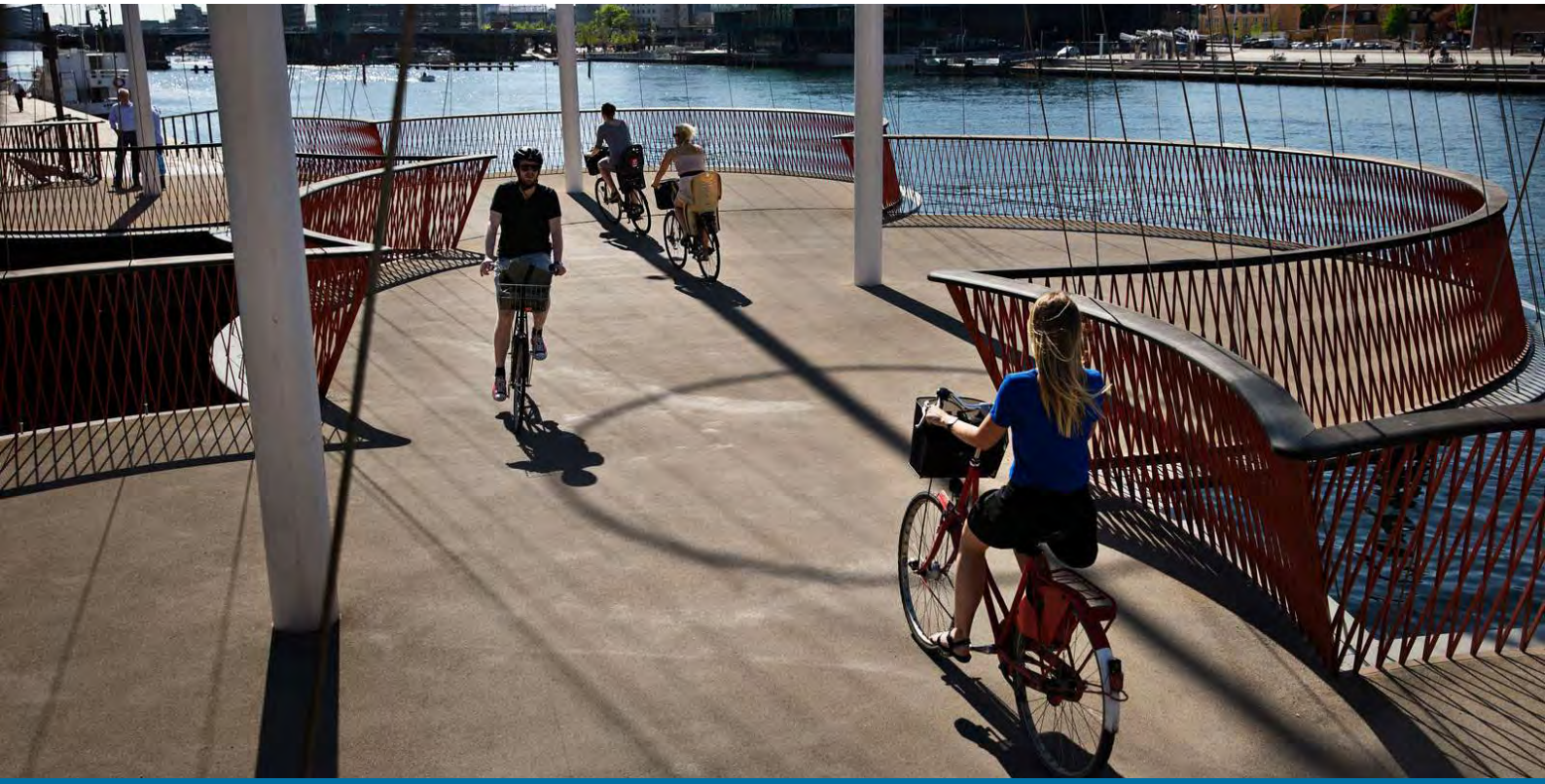
One Health EJP Annual Scientific Meeting 2021 9-11 June in Copenhagen, Denmark and online



Abstract Book

of the 3rd Annual Scientific Meeting of the One Health EJP

Hosted by Statens Serum Institut and
National Food Institute at the Technical University of Denmark

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Contents

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Confronting AMR in times of a pandemic: A global survey on the impacts of COVID-19 on AMR Surveillance, Prevention and Control

Sara Tomczyk

Dr. Sara Tomczyk is with the Robert Koch Institute's Unit on Healthcare-associated Infections, Surveillance of Antibiotic Resistance and Consumption in Berlin, Germany. She leads the unit's international team including their work as the coordinator of the WHO AMR Surveillance and Quality Assessment Collaborating Centres Network. Prior to this, she has worked on a range of other IPC/AMR-related research and outbreak response teams internationally including several years with the WHO IPC Global Hub. She completed the CDC Epidemic Intelligence Service and Preventive Medicine Residency and has a Masters in Epidemiology from the London School of Hygiene and Tropical Medicine.

25 Years DANMAP – past, present and future aspects of an integrated approach to AMR and AMU surveillance

Berit Müller-Pebody

Dr. Berit Müller-Pebody is an Infectious Disease Epidemiologist and Chief Consultant at the AMR Reference Laboratory at the Statens Serum Institut, Copenhagen, Denmark. Berit has a special interest in the surveillance and research of antimicrobial resistance and prescribing, One Health initiatives and data visualisation & linkage methods. She is member of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme's (DANMAP) Steering Committee.

Birgitte Borck Høg

Dr. Birgitte Borck Høg works as Senior Academic Officer at the Division for Global Surveillance, National Food Institute, Technical University of Denmark. Birgitte has a special interest in One Health initiatives and integrated approaches to surveillance. In her work, she has focus on data quality and assurance, multi-sectorial collaboration, as well as communication of results to different audiences. She has worked with different aspects of monitoring and surveillance of zoonoses, including the national control programmes for Salmonella and Campylobacter. Since 2011, she has worked with the Danish integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP, where she is part of the editorial team and co-author of the DANMAP report, and member of the DANMAP Steering Committee.

Important One Health aspects of azole-resistant Aspergillus fumigatus

Maiken Cavling Arendrup

Prof. Arendrup is Professor at the University Hospital Rigshospitalet in Copenhagen and the Head of the Mycology Unit at Statens Serum Institut, Copenhagen, where she is responsible for the fungal laboratory, which receives 13,000 routine and reference samples per year for culture, susceptibility testing, antigen- and antibody-detection, and PCR, as well as for the national surveillance programmes of candidaemia and of azole resistance in Aspergillus. Prof. Arendrup was the founder of the Nordic Society of Medical Mycology (NSMM). She is chair of the EUCAST Antifungal Susceptibility Testing Subcommittee Steering Committee, and head of the EUCAST Development Laboratory for fungi. Prof. Arendrup has authored approx. 250 publications in international journals and as book chapters. Her main research interests include the epidemiology, susceptibility, breakpoint development, diagnostics and treatment of fungal infections.

Rasmus Krøger Hare

Rasmus Hare completed his Master's in Biotechnology at the Technical University of Denmark before joining the mycology unit at Statens Serum Institut, Copenhagen Denmark. For >10 years he has been responsible for development and implementation of molecular assays for the diagnosis of superficial and complicated invasive fungal infections. During this period, he completed a PhD on antifungal drug resistance, involving a thorough understanding of resistance mechanisms and genotyping. This involved research visits at the esteemed centres of PHRI (now Rutgers, New Jersey, USA) and Canisius Wilhelmina Hospital (Nijmegen, Holland). He is a Board member and web-moderator of the Nordic Society for Medical Mycology.

One Health, but more than one strain: the case of Klebsiella

Sylvain Brisse

Sylvain Brisse is Research Director at Institut Pasteur and the Head of the research Unit Biodiversity and Epidemiology of Bacterial Pathogens. He is also the Director of two French National Reference Centers, in charge of the microbiological surveillance of diphtheria and whooping cough. He also acts as Director of the Biological Resource Center of Institut Pasteur. Previously he worked on Trypanosoma cruzi and Chagas disease in Bolivia, obtained his PhD in tropical parasitology and evolutionary genetics in Montpellier University, held a 5-year postdoctoral position in clinical microbiology in Utrecht, Netherlands, and headed a core facility for genotyping and genomic studies of microbial pathogens in Institut Pasteur. His research interests include the population biology and evolution of pathogenic microbial species, and their applications in epidemiological surveillance, diagnostics and public health. His main focus is on the multidrug resistant pathogen Klebsiella pneumoniae and on Bordetella pertussis and Corynebacterium diphtheriae. He also develops and maintains widely adopted strain nomenclatures that allow global and cross-sectorial tracking of bacterial sublineages.

Ethics and OHEJP research: Supporting researchers when dealing with ethical issues

François Hirsch

Graduated in immunology and in Science & Medical Ethics. He spent 30 years at the French Inserm holding various positions, including Secretary General of the ethics committee and Deputy Director of the Health Technologies Institute. For three years, he was a national expert seconded to the European Commission, where he contributed to the organization of the ethics evaluation of research projects. He is currently a member of the Inserm Ethics Committee, Secretary General of one of the French registered IRBs and of the International Association for Responsible Research In Genome Editing (ARRIGE). He is an ethics evaluator for various EC agencies, a member of the European network of research ethics committees. For many years, he is involved in initiatives aiming at training experts from LMICs in ethics and biomedical research, and at establishing guidelines for the conduct of ethical research with vulnerable populations.

Kate Millar

Kate Millar is Professor of Applied Bioethics and Technology Assessment and Director of the Centre for Applied Bioethics, School of Biosciences and School of Veterinary Medicine and Science. Kate's work focusses on animal, biotechnology and agri-food ethics, the development of ethical frameworks (e.g. the Ethical Matrix), publics / stakeholder engagement and biosciences research ethics training approaches. Kate has extensive international and UK research experience with current work funded by the Bill and Melinda Gates Foundation (BMGF), EC H2020, The Wellcome Trust, UK BBSRC and EPSRC, as well as charities such as the 3Rs organisation, FRAME. Kate is President of the European Society for Agricultural and Food Ethics (EurSafe). She is on the Editorial Board of Food Ethics (Springer). She serves on a number of UK and international ethics advisory committees and international research funding boards. She was recently appointed as a visiting academic at Centre of Bioethics in Southern and Eastern Africa-(CEBESA), College of Medicine, University of Malawi.

What will cause the next pandemic?

Mark Woolhouse

Mark Woolhouse has been Professor of Infectious Disease Epidemiology at the University of Edinburgh since 1997. He studied biology at the Universities of Oxford and York and Queen's in Canada, then held Research Fellowships at the University of Zimbabwe, Imperial College London and Oxford. His research interests concern the population dynamics of pathogens at the human-animal interface, especially those associated with antimicrobial resistance (AMR) and emerging infectious diseases. He regularly advises governments and national and international agencies. He was awarded an OBE in 2002 and is a Fellow of the Royal Society of Edinburgh, the Academy of Medical Sciences and the African Academy of Sciences.

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[O1] AN OUTBREAK OF SARS-COV-2 IN MINK AND MINK FARMERS ASSOCIATED WITH COMMUNITY SPREAD, DENMARK, JUNE TO NOVEMBER 2020

Helle Larsen,[1], Jannik Fonager,[2], Frederikke Kristensen Lomholt,[3], Tine Dalby,[4], Guido Benedetti,[3], Brian Kristensen,[5], Tinna Ravnholt Urth,[3], Morten Rasmussen,[6], Maria Lassauniere,[7], Thomas Bruun Rasmussen,[2], Bertel Strandbygaard,[2], Louise Lohse,[2], Manon Chaine,[7], Karina Lauenborg Møller,[2], Ann-Sofie Nicole Berthelsen,[2], Sarah Kristine Nørgaard,[3], Ute W. Sønksen,[8], Anette Boklund,[9], Anne Sofie Hammer,[10], Graham J. Belsham,[11], Tyra Grove Krause,[2], Sten Mortensen,[12], Anette Bøtner,[13], Anders Fomsgaard,[14], Kåre Mølbak,[15]

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Aim: In June 2020, SARS-CoV-2 began to spread among farmed mink in Denmark. We describe the human outbreaks related to mink farming and the public health response.

Methods: We identified farms with SARS-CoV-2-infected mink, individuals connected to mink farms, and in the community in June–November 2020 (1,2). Whole genome sequencing was undertaken on animal and human samples. Public health response was reviewed (1).

Results: SARS-CoV-2-infected mink were detected in a total of 290 of 1,147 mink farms by December 7, 2020 (3). In North Denmark Region, 27% (95% confidence interval: 25–30) of SARS-CoV-2-strains in humans were mink-associated and 30% (324/1,092) of individuals connected to mink farms tested SARS-CoV-2-PCR-positive (1).

By October, despite a policy of intensive surveillance and enhanced biosecurity, mink on 41 farms in Northern Jutland had been infected, and it was decided to cull all mink on infected and surrounding farms. By November 4, mink on 207 farms had become infected, and the government decided to cull all farmed mink. From the end of November, the number of human mink-associated cases decreased. Since mid-January 2021, none have been detected.

Conclusions: The spread of infection from humans to animals, between farms, or from animals to humans was not controlled until culling of mink was completed. However, following the cull, the mink-associated variants disappeared among humans, indicating that their continued transmission was sustained by the animal reservoir. A One-Health approach is paramount to address the risk that farmed mink may pose for SARS-CoV-2 control.

[O2] THE EMERGENCE OF CHRONIC WASTING DISEASE IN EUROPE: A ONE HEALTH PERSPECTIVE

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Aim: To provide an overview and update of CWD in Europe an address one health implications.

Methods: Compilation of latest published knowledge supplemented with own data.

Results: CWD is an emerging and evolving complex of prion diseases, with different epidemiological, molecular and phenotypical presentations. An outbreak of contagious CWD affected wild reindeer in Norway (1). A variant of CWD, characterized as atypical and likely sporadic has been detected in old moose (2) and a red deer (Cervus elaphus). CWD prion strains in Norwegian cervids are distinct from those in NA (3). Strains in reindeer were shown to be different from those in moose in Norway, further, different strains were revealed among the moose (3).

Conclusions: CWD impacts the health and management of cervid populations, has important economic and ecologic consequences and raises public health concerns. Fortunately, to date CWD has not been shown to affect humans. Nonetheless, the rise of new or previously unrecognized prion strains added to the limited knowledge on their host-pathogen interactions indicate the importance of monitoring and assessing of their zoonotic potential. More knowledge on the ecology of CWD in Europe is necessary to better inform management.

References:
Benestad, S.L., et al. First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. Vet Res, 2016, 47:88.

Pirisinu, L. et al. Novel type of chronic wasting disease detected in moose (Alces alces), Norway. EID, 2018, 24 (12): 2210-2218.

Nonno, R. et al. Studies in bank voles reveal strain differences between chronic wasting disease prions from Norway and North America. PNAS, 2020, 117 (49): 31417-31426.

[O3] ONE HEALTH: SETTING UP A RISK ANALYSIS SYSTEM FOR ZOONOSES

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Aim: The goal is to support European countries to improve collaboration between food, environmental, veterinary and public health sectors with respect to risk analysis (signalling, risk-assessment, response and control) of new and (re)emerging zoonoses and antimicrobial resistance. Since no country is the same, there is no blue-print for such a One Health risk analysis system (OH-RAS). Therefore, practical guidelines are under development by the OHEJP project COHESIVE.

Methods: In an iterative process the guidelines are being developed in consultation with experts of different sectors and disciplines. Workshops (live and online) with several assignments have been prominently used. In assorted working groups various parts of the guidelines are written in a collaborative manner. Internal and external review will follow.

Results: The focus of the guidelines is to give practical advice on the implementation and operation processes. It is divided in a stepwise approach on HOW to set up a OH-RAS and in WHAT to organize. Special attention is given to identified barriers that occur in those processes such as information sharing, trust, political will and communication. Tools, tips and tricks are provided. The guidelines are broadly applicable.

Conclusions: The COVID-crisis made clear that it is important to be prepared, including zoonoses. Even when it is unclear what exactly is going to happen, it is crucial to have a system in place in which roles, responsibilities and processes are defined and where people across sectors know and trust each other. These guidelines can contribute to setting up or strengthening such national OH-RAS. The COVID-crisis provides also momentum to obtain political will in order to implement a OHRAS, where in peace time this might be a barrier

[O4] A USER-FRIENDLY DECISION SUPPORT TOOL TO ASSIST ONE-HEALTH RISK ASSESSORS

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Aim: This project's aim is to support the decision-making between different methods of risk assessment using an intuitive interactive decision tree. We aim to describe a diverse range of approaches and provide links to published tools, guidance and examples that are relevant to the user's set of responses.

Methods: A workshop with participants from a range of One-Health backgrounds provided themes on which to assess the user's risk assessment requirements. These were used to devise questions. A tool was then written in html and javascript to make these questions interactive, providing follow-up questions based on answers to previous questions converging, with each question, on one or two appropriate approaches. A literature search was conducted using the snowball method to find tools, guidance and publications related to each risk assessment approach.

Results: Six themes were elucidated to characterise the user's requirements. Fifteen distinct approaches were then determined, with 30 related tools, guidance documents and publications to support them. These approaches cover qualitative risk methodologies, besbpoke models, prioritisation tools, and quantitative microbial cost-benefit assessments. The tool is open-access and live on the OH-EJP website.

Conclusions: The tool is a valuable resource for risk assessors new to the field of One-Health, or who are unfamiliar with the bredth of available approaches. While it is now open for all to access, we are currently seeking feedback from potential users to improve both the content and the user interface.

Funding: This study was partially funded by the Horizon 2020 grant no. 773830, which was matched by the Department for Environment, Food and Rural Affairs (UK)

[O5] MAPPING KNOWLEDGE GAPS OF SOURCE ATTRIBUTION METHODS AND SOURCES FOR ZOONOSES AND RESISTANT BACTERIA USING A RAPID REVIEW APPROACH

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Aim: Get an overview of knowledge gaps with respect to sources and methods considered in source attribution approaches concerning Salmonella, Campylobacter, VTEC/STEC and ESBL/AmpC E.coli.

Methods: A rapid review of the literature using boolean keyword phrases in two data bases for scientific publications (Scopus and Web of Science) was performed, looking for research articles of the last 30 years (from 1990 to 2020). Results of that search were subsequently tagged using the categories “Source”, “Method”, “Organism” and “Location”. Each of these tags was subdivided further into certain values. E.g. ”Source” could be attributed values like ”production animal-cattle”, ”production animal-chicken”, ”production animal-dairy cow”, etc. The tagged results of the rapid review was read into the statistical software R and descriptive statistics were created, among them heatmaps indicating potential knowledge gaps.

Results: Throughout the microorganisms and sources considered, the methods risk assessment, as well as meta-analysis, systematic reviews and time series analysis appear to be underused methodologies for source attribution. Flour is a potentially under-studied source. As regards wild animals, they are hardly considered as source in source attribution studies for all bacterial species. For Salmonella additionally the water environment shows gaps in terms of studies. For Campylobacter additionally human-to-human infection and companion animals are rarely considered as sources in our corpus of literature. For VTEC/STEC one can add human-to-human infection and travel to the sources not considered probably due to knowledge gaps. For AMR E. coli there is so little information available, that every source-method combination can be considered to reflect a knowledge gap.

Conclusions: The rapid review performed gives a broad overview of existing literature and potential knowledge gaps in terms of not considered sources and source attribution methods.

Funding acknowledgement: This work was supported by funding from the European Union’s Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

[O6] TREND REVERSAL IN HUMAN INFECTIONS WITH SALMONELLA ENTERITIDIS IN THE NETHERLANDS (2005 – 2019)

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Aim: Human salmonellosis remains the second most common zoonosis in the EU despite a decreasing trend since 2008. In recent years, this decreasing trend has stagnated in the EU, particularly for *Salmonella* Enteritidis (SE) (1). The aim of this study is to explore temporal patterns (trends, seasonality, periodicity) in reported human SE infections in the Netherlands.

Methods: 5,478 records of culture-confirmed human SE infections in the Netherlands from 2005 to 2019 were retrieved from national surveillance registries. Negative-binomial regression with restricted cubic splines was used to assess temporal patterns in SE incidence while controlling for sociodemographic variables (age, gender, urbanization, and socioeconomic status), outbreaks, season, and long-term trends.

Results: SE incidence exhibits a multi-annual trend, with a decreased incidence in earlier years and increased incidence from 2010 onwards. Seasonality was significant, peaking in the summer. Increased SE incidence was significantly associated with individuals aged 0-4, 5-14, and 15-59 versus ≥60 years [IRR(95%CI): 2.92(2.60 – 3.27), 1.85(1.67 – 2.05), 1.09(1.00 – 1.18), respectively], female versus male [IRR(95%CI): 1.07(1.00 – 1.13)], and living in intermediate and urban areas versus rural areas [IRR(95%CI): 3.74(3.45 – 4.06), 1.26(1.14 – 1.38), respectively].

Conclusions: In the Netherlands, SE incidence showed an increasing trend since 2010, with infection risk depending mostly on the season, younger individuals, females, and those living in intermediate and urban areas. Identifying temporal patterns for foodborne pathogens is an important step for defining options for control. This study indicates potential targets to further identify possible determinants and interventions to re-establish the pre-2010 decreasing trend in human salmonellosis.

Reference:
EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2019. The European Union One Health 2018 Zoonoses Report. EFSA Journal 2019; 17:5926, 276 pp.

Funding: This study was performed as part of the project Assessing Determinants Of the Non-Decreasing Incidence of Salmonella ("ADONIS") and funded through the One Health European Joint Programme by the EU's Horizon-2020 Research and Innovation Programme (grant nr: 773830).

[O7] TRACING THE EVOLUTION OF THE EMERGING MULTIDRUG-RESISTANT SALMONELLA ENTERICA SEROTYPE 4,[5],12:I:- SEQUENCE TYPE 34

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Aim: Multidrug-resistant *Salmonella enterica* serotype 4,[5],12:i:- sequence type (ST)34 is currently one of the most common *Salmonella* clones identified around the world. The public health risk posed by this major foodborne pathogen is aggravated by its carriage of plasmid-mediated resistance to clinically important antimicrobials. Here, we aimed to determine its origin in the United States.

Methods: Publicly available sequences of S. 4,[5],12:i:- ST34 isolates collected during 2008-2017 from USA (n=741) and Europe (n=660) were used. A Bayesian modeling approach was applied on ten subsets (n=112 each) to reconstruct a time-scaled phylogeny with a discrete trait geospatial model. In addition, the presence of plasmid mediated resistance genes (including to clinically important antimicrobials such as quinolones and colistin) was evaluated.

Results: The ST34 clone circulating in USA was introduced on multiple occasions from Europe at the beginning of the 21st century. Combining the directionality of transmission inferred by the model with travel information enabled to demonstrate that trans-Atlantic travelers were likely to be one of the vehicles for introduction. In addition, we found multiple plasmid mediated resistance genes in European and USA sequences, as well as mcr genes conferring resistance to colistin (n=5) only in the European sequences.

Conclusions: This study demonstrated the evolution and epidemiological dynamics of S. 4,[5],12:i:- ST34. The presence of plasmid mediated resistance genes to key antimicrobial classes further highlights its potential risk for public health, and emphasizes the need for developing robust surveillance and mitigation programs for such highly important transboundary multidrug-resistance foodborne Salmonella clones.

[O8] IS SYNDROMIC APPROACH WELL SUITED FOR FOOD-BORNE DISEASES SURVEILLANCE? IMPLICATION FOR SALMONELLOSIS SURVEILLANCE AND PREVENTION IN FRANCE WITH A ONE HEALTH PERSPECTIVE

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Aim: Syndromic surveillance has proven to be a major breakthrough for near real-time disease surveillance in public health. It relies on existing non-specific data, usually collected for other purposes. This work introduces a first approach to surveillance of food-borne zoonosis outbreaks through monitoring of *Salmonella* across the food chain in France, connecting animal and human health data in a One Health perspective.

Methods: This study is based on five datasets, identified through a mapping of the farm to fork flow chart and its surveillance systems. Evaluated datasets covered on-farm cattle mortality, laboratory *Salmonella* isolations along the food chain and entries to public emergency services from 2011 to 2018. Nineteen weekly time series at national scale extracted from these datasets were retrospectively analysed. We used five anomaly detection algorithms (Holt-Winters, Historical Limits, Exponentially Weighted Moving Average, Shewhart and Cumulative Sum) to identify abnormal excess events over the last three years for each time series.

Results: Considering time series individually, some excess events were reported simultaneously by numerous algorithms. Likewise, several of them were measured concurrently in time series from either multiple animal datasets or both animal and human health datasets.

Conclusions: Concomitant occurences of excess events in multiple datasets support further field investigations. This study demonstrated the potential of syndromic surveillance to monitor food-borne diseases in a One Health approach. Nevertheless, data quality and real-time data collection are key to a reliable syndromic surveillance. Temporal and geographical resolutions also affect anomaly detection.

Funding: This work was done as part of the NOVA project under the One Health EJP, a European Union's Horizon 2020 Research and Innovation Program under Grant Agreement 773830

[O9] MOLECULAR METHOD FOR DETECTION OF TOXOPLASMA GONDII OOCYSTS IN LEAFY-GREEN VEGETABLES: METHOD SELECTION, VALIDATION AND SOP DEVELOPMENT

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Aim: Toxoplasma gondii is a zoonotic pathogen with up to 60% of acquired infections associated to foodborne transmission. Consumption of unwashed raw fresh produce contaminated with the environmentally resistant T. gondii oocysts is one of the possible sources of infection. However, the relative importance of fresh produce consumption for human infections is unknown, partly due to lack of standardized detection method(s). The aim of our work was to establish a standard operating procedure (SOP) for molecular detection of T. gondii contamination in leafy green salads.

Methods: An extensive literature review and multi-attribute assessment of the molecular methods described and currently used to detect T. gondii oocysts was conducted (1, 2). Based on the available literature, a comparative experimental work, using artificially spiked salad, was performed at two partner institutes for all analytical steps necessary for the SOP. Effects of different equipment, DNA extraction kits, qPCR platforms reagents and platform were evaluated.

Results: A rapid and efficient DNA extraction method relying on mechanical oocysts lysis combined with a specific highly sensitive qualitative multiplex qPCR assay was selected showing overall a limit of detection of one T. gondii oocyst/3 grams of salad. The method was also reliable in terms of robustness, reproducibility, repeatability, as well as availability and costs of reagents and equipment.

Conclusions: A preliminary SOP was drafted and, supported by video tutorials, is currently under implementation among OHEJP JRP TOXOSOURCES partners. Following validation by a ring trial, the SOP will be used for a multi-center pilot survey on ready-to-eat salads.

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Lalle et al., 2020 https://doi.org/10.5281/zenodo.3778719
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[O10] DEVELOPMENT OF A NEXT-GENERATION SEQUENCING-BASED TYPING METHOD FOR TOXOPLASMA GONDII FOR EUROPEAN NEEDS

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Aim: *Toxoplasma gondii* is a zoonotic protozoan parasite with a largely clonal population structure in Europe. The available PCR-based typing methods have a limited ability to discriminate between the strains circulating in the European context. We therefore aim to establish a next-generation sequencing (NGS)-based typing method with a higher typing resolution among closely related type II *T. gondii* strains.

Methods: Whole genome sequences of more than 80 *T. gondii* isolates including sequences of 60 European field isolates were assessed by whole genome sequencing (WGS). Using a bioinformatics pipeline, highly polymorphic regions were identified. These regions showed a considerable number of single nucleotide polymorphisms (SNPs), insertions and deletions (INDELS). The regions are evaluated for suitability for establishment of a novel typing method.

Results: The identified loci have potential for typing because they are located on 11 of the 14 *T. gondii* chromosomes. As the next step, an amplicon-based enrichment of the promising loci will be established. In a final step, NGS is going to be used to assess amplified typing loci.

Conclusions: The results are promising for establishing a novel typing method with the typing power that is needed in the European One Health setting. The novel method is expected to be useful to better understand the molecular epidemiology of the parasite in Europe, to trace infection sources in outbreaks, as well as to detect introduction of exotic strains or emergence of recombinant strains.

Funding: This work was done as part of TOXOSOURCES project, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

[O11] BETWEEN BATCH VARIABILITY OF HEPATITIS E VIRUS INFECTIONS IN PIGS AT SLAUGHTER POINTS TO FUTURE FARM INTERVENTIONS

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Aim: Zoonotic hepatitis E viruses (HEV) of genotype 3, 4 can cause hepatitis in humans. Humans can become infected by a.o. consumption of undercooked pork. Cross-sectional studies found HEV on most pig farms globally, yet within-farm seroprevalence estimates vary considerably. As pigs are kept in separate units within farms with little contact between units, cross-sectional seroprevalence studies may not give a representative estimate of the within-farm seroprevalence. Therefore, we investigated the variability of HEV infections within farms, between batches of slaughter pigs.

Methods: A repeated sampling study was conducted. From 210 farms, 4 or more batches of slaughter pigs were sampled by randomly collecting ~6 blood samples per batch, giving ~12.000 samples in total. Individual sera were tested for HEV antibodies and pooled sera per batch were tested for HEV-RNA by RT-PCR.

Results: Each farm had seropositive animals, with a median within-farm seroprevalence of 77.8% (IQR: 66.7 – 87.0%) and a mean fraction of PCR positive batches of 40% (IQR: 21.0 – 59.0%). The variability in seroprevalence between farms was low. However, we found variability in serological and pooled PCR results between batches. Namely, 8 farms delivered 30 to 50% batches free of HEV and 20% of farms produced at least one batch free from HEV.

Conclusions: The presence of HEV free batches of fattening pigs shows that transmission of HEV between units on farms may be limited and indicates that there is a potential to intervene and reduce HEV in farms.

[O12] PHYLOGENETIC TRACKING OF LA-MRSA ST398 INTRA-FARM TRANSMISSION AMONG ANIMALS, ENVIRONMENT AND HUMANS ON GERMAN DAIRY FARMS

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Aim: Methicillin-resistant *Staphylococcus aureus* (MRSA) are a major threat to human and animal health causing difficult-to-treat infections such as bovine mastitis. The aim of our study was to evaluate the intra-farm transmission of livestock-associated (LA) MRSA sequence type (ST) 398 isolates between animals, humans and the environment on German dairy farms in order to give recommendations for transmission reduction.

Methods: A total of 115 LA-MRSA ST398 isolates originating from quarter milk samples (QMS), bulk tank milk and swabs from calves, heifers, pigs, farm personnel as well as from the environment of six dairy farms were analyzed by whole-genome sequencing and subsequent phylogenetic analyses using core genome multilocus sequence typing.

Results: Phylogenetic clusters of high allelic identity were detected on all investigated dairy farms suggesting a MRSA transmission across animals, environment and/or humans. On one farm, closely related isolates in QMS, suckers of calf feeders and nasal cavities of calves indicate a transmission by feeding MRSA-contaminated milk and improper hygiene procedures in relation to calf feeding. Detection of related MRSA isolates in QMS and teat cups (4/6 farms) or QMS and human samples (3/4 farms) pointed out that MRSA may be transferred between cows during the milking process and that there is a risk of zoonotic transmission to humans.

Conclusions: LA-MRSA ST398 isolates may be transmitted between animals, humans and the environment on dairy farms. Milking time hygiene and other internal biosecurity measures on farms and pre-treatment of milk before feeding it to calves may reduce the risk of MRSA transmission.

[O13] COMPARATIVE ANALYSIS OF THE RESISTOME AND THE MOBILOME OF ESTUARINE AQUACULTURES AND OTHER AQUATIC ENVIRONMENTS

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Aim: Rivers and estuaries are generally considered to be a natural reservoir of antibiotic resistance genes (ARGs) that can be transmitted to terrestrial animals and to the environment. Furthermore, human activities such as aquaculture industry in those regions contribute to the increase of ARGs. In this scenario, we aimed at performing a comparative analysis of the ARGs metagenomic profiling of sediments of intensive aquaculture farms with other aquatic environments

Methods: High-throughput sequencing-based metagenomics was used to characterize the wide-spectrum profile of ARGs in sediments from bivalve and sea bass aquacultures, and compare with other aquatic environments. Analysis of sequences was performed using the MG-RAST pipeline. We searched for ARGs orthologues, classified into antibiotic resistance families, and also for relaxases, transposases and integrases, indicative of mobile genetic elements (MGEs), using the Resfams and MUSTAD databases.

Results: We characterize both the diversity of antibiotic resistance genes and estimate the number of mobile genetic elements in all microbiomes of our dataset. Our preliminary results show that the microbiomes of natural environments are very rich in ARG and MGE. Yet, the ratio of MGE/ARG is higher in estuarine aquacultures, suggesting that the capability of resistance determinants transfer is higher in these regions subjected to anthropogenic activities.

Conclusions: Although samples of environmental microbiomes from untouched environments like Antarctica share a very rich and diverse repertoire of ARGs and high number of horizontal gene transfer elements, microbiomes belonging to sediments from river aquaculture, close to an urban location show a greater epidemic potential of ARGs spreading.

[O14] EVALUATION OF ENVIRONMENTAL WATERS AND SEWAGE SOURCES FOR THE PRESENCE OF ANTIMICROBIAL RESISTANT ENTEROBACTERALES IN IRELAND

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Aim: Antimicrobial resistance (AMR) is a significant global 'One Health' challenge. The aim of this study was to examine sewage sources and water bodies for the presence of antimicrobial resistant Enterobacterales.

Methods: Samples were collected across four local authority areas in the West, East and South of Ireland between July 2019 and November 2020. This included 118 water (30L) and 36 sewage (200mL) samples. Waters were filtered using the CapE method [1], followed by enrichment and subsequent culturing on selective agars (CHROMagar mSuperCARBA and Brilliance ESBL agar). Sewage samples were directly cultured on these agars. Colonies were identified by MALDI-TOF and antimicrobial susceptibility testing was performed following EUCAST criteria. Selected isolates were examined fro $bla_{\text{CTX-M}}$, bla_{VIM}, bla_{IMP}, $bla_{\text{OXA-48}}$, bla_{NDM} and bla_{KPC} by real time PCR.

Results: A total of 18 water and 5 sewage samples harbored one or more carbapenemase producing Enterobacterales (CPE). The most common carbapenemase gene detected was $bla_{\text{OXA-48}}$ (n=18), followed by bla_{NDM} (n=13) and bla_{KPC} (n=4). Widespread dissemination of extended spectrum beta-lactamase (ESBL) producers was evident in 93/118 (79%) waters and 18/36 (50%) sewage samples. Many of these samples comprised of more than one ESBL producer, which encompassed 186 Enterobacterales that harbored $bla_{\text{CTX-M-group1}}$ and 59 that contained $bla_{\text{CTX-M-group9}}$.

Conclusions: Carbapenemase producing Enterobacterales are considered a public health emergency in Ireland and so their detection in the environment is a significant public health concern. These results highlight the need for regular monitoring of the aquatic environment for the presence of AMR to inform policies to protect public health.

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[O15] ASSESING VARIABILITY OF INCX1 PLASMIDS FROM S. ENTERITIDIS ISOLATES IN SPAIN

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Aim: Horizontal transfer, often involving plasmids, is a major mechanism of antimicrobial resistance (AMR) transmission [1]. Interestingly, *S.* Enteritidis, the most frequent serotype in animal and human sources in the European Union [2], is not typically associated with multidrug resistance phenotypes [3], what could be linked with different dynamics regarding plasmid uptake. The purpose of this study was to characterize plasmids circulating in *S.* Enteritidis strains from multiple sources and locations in Spain.

Methods: Overall, 179 *S.* Enteritidis isolates retrieved in 2004-2019 from several sources (domestic and wild animals, food and environment) were sequenced with Illumina technology. Spades was used for genome assembling. AMR genes and plasmid replicons were identified with ResFinder and PlasmidFinder, respectively. Plasmids sequences were built with Recycler and compared with Blast. Roary was used for pangenome analysis.

Results: IncX1 replicons were identified in 27/179 strains but the plasmid sequence of 22 could be obtained. 5 putative IncX1 plasmid groups (plasmids sharing the same genes and highly similar sequences) were identified in more than one strain, two of which were associated with resistance genes: group 1 carrying *aadA1*, *sul1*, *tet(A)* and *dfrA1* in 4 laying hens strains from two distant Spanish regions and time periods (2008-2018); and group 2 carrying a *blaTEM-1B* gene found in strains from egg and laying hen samples collected 10 years apart.

Conclusions: Our comparative genomic analysis revealed the existence of similar plasmids in epidemiologically unrelated *S.* Enteritidis strains present in animals and food in Spain, suggesting their sustained circulation although at relatively low levels.

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[O16] DIFFERENT SEQUENCING AND ASSEMBLY APPROACHES INFLUENCING THE DETECTION OF PLASMIDS AND ANTIMICROBIAL RESISTANCE GENES IN COMMENSAL ESCHERICHIA COLI

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Aim: We investigated different sequencing and assembling methods regarding their ability to detect resistance genes and predict the association to mobile genetic elements as plasmids in *E. coli.*

Methods: Five *E. coli* isolates with distinct resistance profiles and diverse plasmid contents were characterized in detail by antimicrobial susceptibility testing, *XbaI*-/ S1-PFGE profiling, and filter mating studies. The biological characteristics of the isolates were compared with *in silico* predictions using different sequencing strategies. Raw reads were generated using the Illumina NextSeq, the long-read systems PacBio Sequel and MinION. The reads were used individually or in combinations to assess appropriate workflows for reliable *in silico*-based typing purposes.

Results: Long-reads alone were shown to be error-prone, affecting the annotation of genes and the prediction of small plasmids. However, their use represented the best option to generate genome-scaffolds. Short-reads were reliable for detecting resistance genes, but were not suitable for linking specific resistance determinants to the chromosome or to plasmid types. Moreover, short-read sequencing missed duplicated resistance genes. The use of hybrid-assemblies led to the best consensus between *in silico* and *in vitro* results, as all duplications and small plasmids were predicted and provided closed plasmid for allmost all extrachromosomal elements.

Conclusions: Hybrid assembling provided a thorough overview on extrachromosomal DNA elements, associated with antimicrobial resistances and detailed insights into the genomes genetic composition. Overall, it will be worth extending the routine sequence diagnostic to hybrid sequencing, when a reference-grade complete bacterial genome is aimed, or extrachromosomal structures needs to be fully understood.

[O17] COLISTIN RESISTANT ENTEROBACTERALES IN THE GENERAL POPULATION AND VETERINARY HEALTHCARE WORKERS: FIRST DISCOVERY OF MCR-8 IN THE NETHERLANDS

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Aim: To investigate risk factors for carriage and molecular characteristics of colistin resistant *Escherichia coli* and *Klebsiella pneumoniae* (ColR-E/K) in the general Dutch population and veterinary healthcare workers.

Methods: Two studies were included, one in the Dutch population at large and one among veterinary workers. Participants sent in a fecal sample and a questionnaire. Samples were selectively enriched with 2 mg/L colistin and cultured on ChromID Colistin R agar and confirmed by broth micro dilution. Mobile colistin resistance (*mcr*)-genes were detected by PCR and plasmids were reconstructed by combining short-read and long-read sequencing data.

Results: Prevalence of ColR-E/K was 5.4% (36/661; 95% CI 4.0-7.5%) in persons from the general population and 8.1% (39/482; 95% CI 6.0-10.9%) in veterinary workers. This difference was not statistically significant (P-value=0.4). *Mcr*-genes were detected in one veterinary technician and five persons from the general population. Four persons carried *mcr-1* in *E. coli*, one person *mcr-8* in *K. pneumoniae* and another person carried *mcr-1* and *mcr-8* in *K. pneumoniae* on distinct plasmids with IncX4, incI1, incI2 and IncFII replicons. Risk factors for ColR-E/K carriage were antibiotic use, hospital visit, travel abroad, and poor kitchen hygiene.

Conclusions: Contact with animals was not an important risk factor for ColR-E/K carriage. However, the emergence of colistin resistance due to *mcr*-genes, including *mcr-8,* in the community poses a potential threat for the treatment of patients. Surveillance of colistin resistance and its underlying mechanisms in humans, livestock and food is important in order to identify emerging trends in time.

Funding: This work was supported by the Dutch Ministry of Health, Welfare and Sport.

[O18] EVALUATION OF THE EFFECT OF COLISTIN REDUCTION IN PIGS ON MCR-1 DETECTION

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Aim: Since the first description of the plasmid-mediated *mcr-1* gene in *Enterobacteraceae*, the European Medicines Agency (EMA) and the Spanish Ministry of Agriculture (MAPA), recommended limiting the use of polymixins in animals, especially in food-producing animals. Consequently, the Spanish Medicines and Healthcare Products Agency created a strategic plan to reduce the consumption of colistin in the pig industry. This study aims to analyze the evolution and current status of the prevalence of *mcr-1* gene in Spanish food-producing pigs population.

Methods: Samples from the caecal contents of healthy pigs (N=244) were sampled at slaughterhouses in different Spanish regions from January to December 2019. Direct DNA extraction from pig caecal samples was carried out coupled by a real-time SYBR® Green I PCR assay for quantitative detection of the *mcr-1* gene. Statistical analysis was performed using T-Test. Data was previously normalised by logarithmic transformation.

Results: From 244 samples, *mcr-1* gene was quantified in 67 (27.5%), with a median of 2.69 Log_{10} copies/mg faeces. Comparing these data with our previous study (Miguela-Villoldo *et al*, 2019), where we analysed the prevalence of colistin resistance in pigs from 2012 to 2017, we noticed a significantly decrease of the *mcr-1* prevalence in pigs from the 70% reached in 2015.

Conclusions: The levels of *mcr-1* show a declining trend that seems to correlate with variations in colistin sales in Spain, reaching similar values to those obtained in 2012 and 2013. So, the EMA recommendations and the Spanish program are playing an effective role in the fight against colistin resistance in pig production.

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[O19] LEAKINESS AT THE HUMAN-ANIMAL INTERFACE IN SOUTHEAST ASIA AND IMPLICATIONS FOR THE SPREAD OF ANTIBIOTIC RESISTANCE

Maya Nadimpalli,[1], Marc Stegger,[2], Roberto Viau,[1], Vuthy Yith Yith,[3], Agathe de Lauzanne,[3], Laurence Borand,[3], Bich-Tram Huynh,[4], Sylvain Brisse,[4], Virginie Passet,[4], Søren Overballe-Petersen,[5], Maliha Aziz,[6], Thong Phe,[7], Bruce A. Hungate,[8], Victor O. Leshyk,[8], Amy J. Pickering,[1], François Gravey,[9], Cindy M. Liu,[6], Timothy J. Johnson,[10], Simon Le Hello,[4], Lance B. Price,[6]

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Aim: International efforts to curb antimicrobial resistance have focused on drug development and limiting unnecessary use. However, in areas where water, sanitation, and hygiene infrastructure are lacking, and where biosecurity in food-animal production is poor, pathogen-flow between humans and animals could exacerbate the emergence and spread of resistant pathogens. Here, we investigated this hypothesis by using a multi-scale, molecular approach to explore the spread of mobile resistance elements between humans and animals in Phnom Penh, Cambodia, a highly leaky urban center in Southeast Asia.

Methods: We used a combination of short- and long-read sequencing to compare the genomes including plasmids and mobile resistance elements among *Escherichia coli* recovered from humans and meat in Cambodia, a country with substantial connectivity between humans and animals, unregulated antibiotic use, and poor environmental controls.

Results: We identified multiple resistance-encoding plasmids and a novel $bla_{CTX\text{-}M}$ and *qnrS1*-encoding transposon, that were widely dispersed in both humans and animals, a phenomenon rarely observed in high-income settings. Importantly, analyses showed that mobilization of the transposon as well as plasmid sharing across hosts was not exclusively driven by the zoonotic transmission of specific bacterial clones; rather, frequent mixing of host-adapted strains likely allowed for the uptake of each plasmid into diverse genetic contexts.

Conclusions: Our findings strongly suggest that the lack of environmental controls along with widespread antimicrobial use is leading to the dissemination of novel resistance elements and the evolution of antimicrobial-resistant human pathogens in Southeast Asia. Thus, our findings indicate that antibiotic stewardship must be complemented by plugging leaks at human-animal interfaces as a critical part of addressing antimicrobial resistance in low and middle-income countries. In addition to stemming the circular flow of antimicrobial-resistant bacteria, infrastructural WASH and biosecurity improvements will likely also be critical in reducing disease, and thereby, antibiotic demand, in both humans and animals.

[O20] MAPPING THE CURRENT EXISTING HAZARDS' CONTROL PROGRAM AND STRATEGIES IN THE VARIOUS SECTORS AND TRACTS OF THE ANIMAL-ENVIRONMENT-FOOD-HUMAN CHAIN IN EU

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Aim: This activity is a part of OHEJP DISCOVER WP5T2 which aims to map and describe existing control programs implemented after 2003 for *Salmonella, Campylobacter,* VTEC/STEC, and antimicrobial resistance (AMR) at the EU and national level. The control programs and their strategies in various sectors of the animal-environment-food-human chain is collected by a set of activities: A structured scientific literature review, expert surveys, and review of grey literature. This presentation will revolve around control programs and strategies extracted from manuscripts by a scientific structured literature review.

Methods: The scientific structured literature review was completed in three databases (PubMed, Scopus, and Web of Science) with a detailed search strategy. Inclusion criteria concerning the zoonotic agent, geographical area, control program, time-period and the objective were applied with the purpose to exclude irrelevant papers.

Results: The total number of retrieved papers for each zoonotic agent were 2,957 with AMR, 2,005 with *Salmonella,* 870 with *Campylobacter* and 377 papers with VTEC/STEC. Based on title and abstract screening, 98 VTEC/STEC, 214 *Campylobacter* and 666 AMR papers set the basis for analysis of control programs within EU/EEA level. The screening of *Salmonella* papers and further analysis of the papers are an ongoing process. The overall characteristics of the retrieved papers describe control programs and interventions with a focus on the primary production level for poultry, swine, and cattle.

Conclusions: The scientific structured literature review will provide an output to a graphical visualisation of knowledge gaps indicating where interventions can be strengthened, introduced, or recommended.

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[O21] OH-HARMONY-CAP: DEVELOPMENT OF AN OHLABCAP TOOL

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Aim: The aim of Work-Package (WP) 2 of One Health (OH) EJP project OH-Harmony-CAP (One Health Harmonisation of Protocols for the Detection of Foodborne Pathogens and AMR Determinants) is to collect information and describe the current laboratory capabilities, capacities and interoperability by providing an in depth OHLabCap survey on both the National Reference Laboratory (NRL) and the primary diagnostic level. In order, to create an OHLabCAP tool across the One Health sectors, a pilot survey was tested and analysed.

Methods: The pilot survey included 63 questions covering three targets: capability, capacity, and interoperability. By using the EU Survey tool the pilot survey was distributed to 46 OH-Harmony-Cap participants representing 15 institutes and laboratories in 11 countries in 2020. The pilot survey covered six priority bacteria and ten priority parasites together with the antimicrobial resistance (AMR) testing of *Salmonella* and *Campylobacter*.

Results: The pilot survey results were analysed by applying "scoring options", similar to the EULabCAP survey, and compiled indicators that were scored across three dimensions: primary diagnostic testing, NRL services, and interoperability and communication. Several survey issues were identified e.g. the length of the survey, order of options, missing options, terminology, and scoring challenges.

Conclusions: The pilot testing proved highly useful. The results illustrated not only the complexity of the OH fields and the need to address several issues but importantly, to include *adaptability* as an additional fourth target in the preparation of the OHLabCap instrument. Moreover, the pilot also indicated that the top five prioritized parasites will be sufficient to be included in the OHLapCap tool.

[O22] PASTORALSCAPE: AN ENVIRONMENT-DRIVEN MODEL OF VACCINATION DECISION MAKING WITHIN PASTORALIST GROUPS IN EAST AFRICA.

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Aim: Economic and cultural resilience among pastoralists in East Africa is threatened by the interconnected forces of climate change, contagious diseases spread and evolving institutional development. A key factor in the resilience of livestock that communities depend on is human decision making regarding vaccination against prevalent diseases such as Rift Valley fever and Contagious Bovine Pleuropneumonia.

Methods: The current PastoralScape agent-based model (ABM) documents a data driven model of herd movement, incidence of Rift Valley fever (RVF) and livestock foraging conditions over an 11-year period (2004 - 2015). Natural and human environments are linked via a human decision sub-model that utilizes memory and 'rationality'. The primary decision of interest is the vaccination of cattle for Rift Valley fever (RVF) and Contagious Bovine Pleuropneumonia (CBPP). The difference in the frequency of vaccinations for each disease provides a means for assessing the effects of memory and 'rationality' on one-time (RVF) and repeated decision-making (CBPP). The ABM introduces a Random Field Ising Model (RFIM) to estimate the binary choice of vaccination.

Results: The interaction between the values of agent memory, agent 'rationality' and fixed values of RFIM parameters provides one indication of the potential of this human decision making paradigm to add value to AMB modeling of human agents. Although convergence of opinion is reach across various agent memory and rationality values, the speed of this convergence differs. The seasonal variation in disease prevalence for RVF implies that the date of vaccination may have an impact on the overall resilience of herds to disease, especially with respect to animals born in the time period since the last vaccination event. In this study we fix all parameters other than the date of vaccination. We perform an ensemble of 100 runs for vaccinations timed at the start of each month.

Conclusions: The development of the PastoralScape model, first introduced by Iles et al. (2020), further details the behavioral utility of the RFIM in capturing human decision making in ABMs. This model is intended to form the basis upon which richer economic and human factor models can be built.

[O23] THE SCOPE OF COMMUNITY ENGAGEMENT (CE) APPROACHES TO TACKLE AMR.

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Aim: Creating behavioral changes with regards to antimicrobial use will safeguard both existing and future treatments but calls for a radical overhaul of traditional antimicrobial resistance (AMR) research. Changing behavior requires engaging with people at a local level to understand their specific experiences and challenges. The Community Engagement (CE) method represents a potential solution that is being utilized by several AMR research groups. If we can synthesize learnings from these interventions, then we can develop a collective understanding of their scope tackle AMR across contexts.

Methods: This presentation describes a synthesis of existing knowledge and experience. It is the work of a GCRF Challenge Cluster funded in 2020 to develop community-led solutions to AMR across the One Health context. We present a macro-level synthesis of group discussions and learnings embedded in recent literature.

Results: CE interventions focus on human health impacts, and the demand-side drivers of AMR. Most strategies favour a mixed method approach generating research data, and community-owned, co-produced outputs. This can facilitate the development of locally specific and meaningful solutions to AMR. However, the specificity of mixed method approaches also present challenges for scaling, sustaining, and evaluating CE activities within the AMR sphere.

Conclusions: CE can facilitate two-way knowledge exchange between research teams and communities which helps contextualise local AMR challenges, and supports the development of meaningful solutions. However, this does not yet extend to the broader One Health dimensions of AMR. We will discuss gaps in the AMR landscape which could benefit from CE interventions and suggest the barriers responsible for the lack of uptake so far.

[O24] PARTICIPATION IN ONE HEALTH NETWORKS AND INVOLVEMENT IN COVID-19 RESPONSE

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Aim: This global study examined associations between being part of a One Health Network (OHN) and involvement in response to COVID-19. Barriers to workforce participation in the pandemic response and the value of OHN activities were studied to identify possible measures to take to strengthen workforce capacity.

Methods: We conducted a global cross-sectional descriptive study using an online survey tool that was distributed in July-August 2020 via OHN listservs, social media, and further sharing. Using a snowball sampling approach, responses were encouraged from individuals across locations, organizations, and sectors.

Results: The sample included 1050 responses from 94 countries from all 6 WHO global regions, across organizations and sectors. Being part of an OHN was positively associated with involvement in the COVID-19 response (odds ratio: 1.8, 95% confidence interval: 1.3 - 2.4). OHN activities that increased public awareness of One Health and were related to networking were perceived as most useful during the pandemic. Overall, 44% of survey respondents who were part of an OHN found OHN activities very or extremely helpful to their COVID-19 response. Lack of opportunities was a commonly reported barrier to workforce participation globally, and lack of funding was a barrier in Africa in particular.

Conclusions: This study provides a snapshot of the multisectoral response to COVID-19 and an assessment of the contribution of OHNs. The lessons learned during this pandemic can be used to identify measures to improve workforce capacity, including OHN activities to strengthen workforce response to future global health challenges.

Funding: This work was supported by the One Health Commission and funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

[O25] LESSONS LEARNED FROM ONE HEALTH PRACTICES IN SWEDEN AND ITALY

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Aim: Swedish and Italian institutes were analysed to provide insight into barriers and opportunities for integrating One Health practices. The two countries showed different approaches to One Health implementation on institutional level, which can provide meaningful information for implementing other One Health activities.

Methods: Semi-structured interviews with experts from Swedish and Italian veterinary, food, public health and environmental agencies were conducted. A thematic content analysis was conducted to analyse the interviews using inductive coding in NVivo.

Results: Between the countries, there are clear differences pertaining to structure like different jurisdictions that affect legislations and responsibilities. Different barriers and opportunities occur in terms of cooperation with the environmental sector and regarding the understanding of One Health. There are legislative-related challenges and barriers for science to policy translations. Similarities between Italy and Sweden were identified as personal challenges during collaborations, and the need to recognising disease outbreaks as an opportunity for integrating One Health.

Conclusions: In both countries, a One Health strategy developed on institutional level can facilitate defining One Health and clarifying roles and responsibilities. To overcome practical challenges, the countries can learn from each other in terms of coordination across disciplines and by implementing training and education for One Health collaboration. Experiences with One Health projects must be disseminated in the scientific field as well as to the public to raise awareness for One Health. Understanding these barriers and opportunities will be beneficial when integrating One Health in Sweden, Italy and in other countries and contexts.

Funding: This project is funded by the One Health EJP

[O26] FOOD PRODUCTS IDENTIFIED AS SOURCE OF A FOODBORNE DISEASE OUTBREAK BY A FAST AND ROBUST LIKELIHOOD ESTIMATION

Jakub Fusiak,[1], Kyrre Kausrud,[2], Marion Gottschald,[3], Dominic Tölle,[3], Marco Ruegen,[3], Birgit Lewicki,[3], Isaak Gerber,[3], Michele Kayser,[4], Alexander Falenski,[1], Armin Weiser,[1], Madelaine Norström,[2], Katja Alt,[5]

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Aim: Identifying a specific product causing a foodborne disease outbreak can be difficult, especially when dealing with a large amount of suspicious food items and weak epidemiological evidence. A previously described likelihood model [1], improved within the OHEJP NOVA project, helps to prioritize food products that should be sampled for laboratory analysis. It is the aim of our study to integrate this approach into state of the art tracing software FoodChain-Lab (FCL) developed at BfR to facilitate outbreak investigations.

Methods: Agile software development.

Results: The model improved by Kausrud et al. in R uses wholesale data, the distribution of disease cases and census data to sort food items by their estimated likelihood to be the source of an outbreak. We developed a fast and secure intuitive software module using the Web Assembly technology allowing professionals to embed the module easily into other applications. Currently, we are integrating the module into the FCL web application for tracing (FCL Web; https://fcl-portal.bfr.berlin) to provide an intuitive and user-friendly solution. This solution combines a simple data input with extended data wrangling to make the calculation of the NOVA model as easy as possible. Since the model can be executed directly inside the web browser and therefore does not rely on any server environment, the possibility of data leakage can be highly reduced.

Conclusions:
The implementation of the advanced likelihood model into FCL Web increase the availability of this model and provides investigators easy, fast and reliable usage to improve outbreak investigation workflows.

References:
Norström M et. al. (2015) An Adjusted Likelihood Ratio Approach Analysing Distribution of Food Products to Assist the Investigation of Foodborne Outbreaks.

[O27] SIMULATION AND IDENTIFICATION OF FOODBORNE OUTBREAKS IN REAL CONSUMER PURCHASE DATA

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Aim: Foodborne outbreaks remain an important cause of morbidity and development of innovative methods for their investigation is therefore important. Consumer purchase data (CPD) gathered by retailers represent such an option. We used real-life consumer purchase data to simulate foodborne outbreaks and examined how the ability to correctly identify the outbreak vehicle varied across outbreak parameters and level of product detail.

Methods: We used CPD from Norway's largest grocery wholesaler, covering five cities in 2019 and constituting 122 million purchases by 920,000 customer IDs. Outbreaks were simulated by defining a contaminated item, an attack rate, and a parameter reflecting the database proportion of each customer's total purchases to address incomplete registration. Using items purchased by infected customers as covariates, we ran case-controlled lasso logistic regression to determine the most significant item. Once an item reached a pre-determined odds ratio (OR), we labeled this as the outbreak vehicle. We also examined model sensitivity to attack rate, database proportion, item frequency, and expiration length.

Results: Overall our model performed very well, correctly identifying the stipulated outbreak vehicle in around 90% of outbreaks, taking on average less than 10 cases to reach the threshold OR of 30. Restricting analysis to product groups reduced the identification rate to around 50%.

Conclusions: Our model is capable of identifying outbreak sources in a large CPD. Analysis of product groups only, comparable to those found in trawling questionnaires, decreases the percentage of outbreaks identified. CPD analysis could greatly assist in a real outbreak scenario, when available.

Funding: Funding: This work is supported by the One Health European Joint Project (OHEJP)

[O28] FOOD SAFETY KNOWLEDGE EXCHANGE (FSKX) FORMAT: A FLEXIBLE EXCHANGE FORMAT FOR (JOINED) MODELS IN THE AREA OF FOOD SAFETY AND ONE HEALTH

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Aim: Efficient knowledge exchange across One Health (OH) sectors is a major challenge for adopting the OH paradigm. Such knowledge include predictive models, data analysis pipelines, or data visualization methods. To support re-use of such knowledge, it is important to develop a modular information exchange format that also support the joining of models from existing model modules. Here, we present the Food Safety Knowledge Exchange (FSKX) format that allows to share various types of models and data. This format is currently extended and tested towards various OH disciplines.

Methods: The FSKX format provides rules on how to annotate and encode models, simulation settings, simulation results, and model related metadata. It extends the existing standards OMEX, SBML, and SED-ML. It allows to share fully annotated models from different scripting languages in one file while providing enough flexibility to incorporate model class-specific annotations.

Results:
The FSKX format facilitates model re-usage and interpretation of simulation results. It provides the basis for online model repositories, like the RAKIP model repository, where models can easily be exchanged across OH sectors. Developers of software tools are now enabled to provide harmonized model import and export features in their software, e.g., in the KNIME-extension "FSK-Lab". Also, the FSKX format allows to easily describe legacy models with only minor changes of the already implemented model.

Conclusions:
The FSKX format allows to efficiently exchange knowledge and create joined models from existing model modules. Thus, it can make the work of researchers and OH professionals more efficient.

Funding:
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[O29] CONTROL OF HUMAN PATHOGENIC MICROORGANISMS IN PLANT PRODUCTION (HUPLANT CONTROL EU COST ACTION CA16110): TERMINOLOGY EXPLAINED WHEN PLANT SCIENCE MEETS FOOD SAFETY

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[8] Zhaw Zurich University of Applied Sciences;
[9] Flanders Research Institute for Agriculture, Fisheries and Food;
[10] Julius Kühn Institute (Jki) - Federal Research Centre for Cultivated Plants;
[11] Wageningen Plant Research

Aim: The EU HUPLANTcontrol COST Action was initiated to promote the cooperation among microbiologists in food, environmental and plant sciences and public health and industrial stakeholders. Scientific insights on behaviour of zoonotic and phytonotic pathogens within the plant microbiome will guide agricultural management practices in order to combat disease outbreaks. It was the objective of HUPLANTcontrol to facilitate the multidisciplinary collaboration by fine-tuning common understanding of used definitions and jargon.

Methods: The HUPLANTcontrol COST Action has initiated a number of workshops to clarify the terminology used in the respective research fields and to discuss the (debatable) definition of human pathogenic microorganisms, hazards, risks and 'good agricultural practices'.

Results:

- The difficulty in defining a (bacterial) species has multiple implications. One is the challenging communication of scientific findings across disciplines, as well as the communication with stakeholders and public health management, lacking the expertise in bacterial systematic or molecular biology.
- It is necessary to invest in hazard identification and characterisation. This increases our knowledge; however, decision-making on food safety should be based on risk, preferably using a holistic risk assessment. This is exemplified in the present debate on the safety assessment (QPS) of *Bacillus thuringiensis* as a biological control agent in plant primary production.
- 'Microbes are not always bad': plants should not be grown in a fully sanitised environment. The plant microbiome may be suppressive to the establishment of human pathogens in fresh produce.

Conclusions: A common language is needed in communication about strategies, costs and benefits of intervention measures to ensure safe plant-based food production.

[O30] AN INVENTORY OF ZOONOTIC AND FOOD-BORNE DISEASE SURVEILLANCE SYSTEMS: EXPANDING THE ONE HEALTH KNOWLEDGE BASE.

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Aim: Effective disease management decision making is often underpinned by surveillance data. For zoonotic and food-borne diseases that benefit from a One Health (OH) approach, data from multiple surveillance systems may be required. However, identifying and accessing these data can be difficult as they are often collected at different governmental levels, presented in different platforms, and published in different languages. To address this problem, we aimed to develop an accessible and searchable inventory of the zoonotic and food-borne disease surveillance systems in existence across Europe.

Methods: We created spreadsheet-based questionnaires to collect uniform data describing relevant surveillance systems from each of the sectors: animal health, public health, and food and feed safety. The variables for inclusion were determined by consensus selection following several rounds of consultation with ORION project members and stakeholders. Final spreadsheets were sent to all project partners for data contribution.

Results: Currently, the inventory contains 62 entries for public health, 149 for animal health and two for food and feed safety, although additional contributions are expected. Data are easily accessible on an interactive web based application, (https://shiny.fli.de/ife-apps/EJPOrion_WP2Epi/), developed using R software and in combination with the “shiny” package. The application includes intuitive search functions that allow for identification and comparison of the surveillance systems in existence for a specified pathogen, across both sectors and countries.

Conclusions: This surveillance inventory facilitates both information accessibility and exchange across sectors and countries for improved OH approaches to disease management. Ongoing maintenance of the inventory will continue in the MATRIX project.

Funding: This work was done as part of ORION project in collaboration with MATRIX project, supported by funding from the European Union’s Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme

No.	Title
P001	TRANSCRIPTOMIC PROFILING ANALYSIS OF CLOSTRIDIUM PERFRINGENS ISOLATES COLLECTED FROM HUMAN AND FOOD POISONING OUTBREAKS
P002	EXPLORING THE EVOLUTIONARY SUCCESS OF THE ANTIBIOTIC-RESISTANT SALMONELLA KENTUCKY ST198
P003	COLISTIN RESISTANCE IN SALMONELLA ENTERITIDIS ST11 IN THE POULTRY INDUSTRY INTERFACE, PORTUGAL 2014-2020
P004	DYNAMICS OF EXTENDEN SPECTRUM BETALACTAMASE RESISTANCE GENE BLASHV-12 IN A LAYING HEN COMMERCIAL FARM
P005	EMERGENCE OF MULTIDRUG RESISTANT SALMONELLA INFANTIS ST32 IN THE POULTRY INDUSTRY, PORTUGAL 2016-2020
P006	A SCOPING REVIEW ON THE IMPACT OF HEAVY METALS IN THE AGRI-FOOD ENVIRONMENT AS A SELECTIVE PRESSURE FOR ANTIMICROBIAL RESISTANCE
P007	DETECTION OF THREE SWINE-RELATED VIRUSES (HEPATITIS E VIRUS - HEV, MAMMALIAN ORTHOREOVIRUS - MRV, AND TORQUE TENO SUS VIRUS - TTSUV) IN THE WILD FAUNA OF NORTHERN ITALY
P008	GAME MEAT OF CULINARY IMPORTANCE AS A POSSIBLE SOURCE OF HEPATITIS E VIRUS (HEV) INFECTION IN POLAND
P009	EDIBLE PORK SLAUGHTER BY-PRODUCTS AS SPORADIC SOURCES OF ZOONOTIC HEPATITIS E VIRUS IN POLAND
P010	EJP-OH-CARE: PILOT STUDIES TO SUPPORT GUIDANCE TO QUALITY ASSURANCE TESTING OF LABORATORY METHODS WITHIN A ONE HEALTH CROSS-SECTORAL FRAMEWORK
P011	ASSESSING CORONAVIRUS RISKS: FEASIBILITY OF PREDICTING TRAITS OF CLINICAL AND EPIDEMIOLOGICAL RELEVANCE FROM SEQUENCE DATA
P012	THE HARMONISED ONE HEALTH EJP DATA MANAGEMENT APPROACH ENSURES THAT THE DATA ARE FAIR (FINDABLE, ACCESSIBLE, INTEROPERABLE AND REUSABLE)
P013	COMPARATIVE STUDY OF BACTERIAL DNA EXTRACTION METHODS ON COMPLEX SAMPLES FROM THE MARINE ENVIRONMENT.
P014	DETERMINATION OF SELECTION PRESSURES IN ENVIRONMENTAL SAMPLES
P015	EUROPANELOH: CONSTRUCTION OF A CARE CATALOG OF BACTERIA FOODBORNE PATHOGENS STRAINS USED AS REFERENCE MATERIALS IN THE EUROPEAN UNION
P016	DEVELOPMENT OF AN APTAMER-BASED TEST FOR TRICHINELLA DETECTION
P017	CAN THE AGRICULTURAL MICROBIOME BE UTILISED FOR SUPPRESSING ZOONOTIC PATHOGENS IN PLANT FOODS?
P018	INTRANASAL INFECTION OF FERRETS WITH SARS-COV-2 AS A MODEL FOR HUMAN AND MINK INFECTION
P019	DISSEMINATION OF ANTIMICROBIAL RESISTANT CLOSTRIDIUM DIFFICILE RT078/ST11 IN AUSTRIA ACROSS THE HUMAN-ANIMAL-WILDLIFE INTERFACE
P020	A BRIEF EXCURSION INTO PARADISE
P021	COMPARATIVE GENOMICS OF CRYPTOSPORIDIUM PARVUM AND GIARDIA DUODENALIS ISOLATES FROM EUROPE: TOWARDS THE DEVELOPMENT OF NOVEL GENOTYPING SCHEMES
P022	LIGHTS AND SHADES IN GENOTYPING: EUROPEAN TOXOPLASMA GONDII NEEDS A CLOSER LOOK USING HARMONISED APPROACHES
P023	FASCIOLA SPECIES INTROGRESSION: JUST A FLUKE OR SOMETHING MORE?
P024	A SEMI-AUTOMATED IN VITRO MODEL TO STUDY THE TRANSMISSION DYNAMICS OF ANTIMICROBIAL RESISTANCE IN THE CHICKEN CAECAL MICROBIAL COMMUNITIES
P025	DIFFERENCES IN CAECAL MICROBIOTA COMPOSITION OF BROILER CHICKENS COLONISED AND NON-COLONISED WITH ESBL-ESCHERICHIA COLI
P026	MEME PROJECT UPDATE ON ECHINOCOCCUS MULTILOCULARIS AND ECHINOCOCCUS GRANULOSUS S.L. IN EUROPE
P027	OCCURRENCE OF TICK-BORNE-ENCEPHALITIS IN THE AUTONOMOUS PROVINCE OF BOLZANO – ITALY: AN EXAMPLE OF ONE HEALTH APPROACH IMPLEMENTATION AT LOCAL LEVEL
P028	ENABLING THE BROAD IMPLEMENTATION OF FULL-LENGTH SEQUENCING FOR IMPROVED SURVEILLANCE OF ANTIMICROBIAL RESISTANCE
P029	IN SILICO STUDY OF IS6110 SEQUENCES ABUNDANCE AND LOCALISATION EVOLUTION IN THREE MAIN MYCOBACTERIUM BOVIS FRENCH GENOTYPES OF ENDEMIC REGIONS
P030	NEW REFERENCE GENOMES OF MYCOBACTERIUM BOVIS ADAPTED A FRENCH GENOTYPE DIVERSITY
P031	MAPPING THE SURVEILLANCE ACTIVITIES FOR FOOD-HAZARDS ACROSS EUROPE
P032	CONCEPTUAL MODEL OF FACTORS IMPACTING GENOMIC CLUSTERING
P033	16S RRNA MICROBIAL COMMUNITY ANALYSIS AND RELATIONSHIP WITH SALMONELLA SUPER SHEDDER STATUS IN PIGS

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[P001] TRANSCRIPTOMIC PROFILING ANALYSIS OF CLOSTRIDIUM PERFRINGENS ISOLATES COLLECTED FROM HUMAN AND FOOD POISONING OUTBREAKS

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Aim: *Clostridium perfringens* is estimated to be the fourth most common bacterial cause of food poisoning outbreaks (FPO) in France and Europe causing a hundred FPO and more than one thousand cases each year. The bacterium is spore-forming anaerobic known to be one of the most significant producer of toxins with more than twenty virulence factors including toxins and digestive enzymes produced. Based upon the production of six major toxins (CPA, CPB, ETX, ITX, CPE and NetB) *C. perfringens* is classified into seven types (A-G). It has been demonstrated that the enterotoxin CPE, encoded is essential to the development of gastroenteritis. Through its complex regulatory system, *C. perfringens* orchestrates the expression of a collection of toxins and extracellular enzymes that are crucial for the development of the disease. In this study, RNA-Seq-based global transcriptome analysis was then performed with the objective to compare the transcriptome under these two conditions.

Methods: Four CPE-positive *C. perfringens* strain (16SBCL940, 17SBCL79) collected from FPO and from human (168-2017 and 197-2019) were grown under vegetative and sporulation conditions.

Results: Many genes involved in amino acid metabolism and carbohydrate degradation were similarly upregulated in all five isolates confirming that sporulation of *C. perfringens* can be triggered by upregulation of genes involved in sugar uptake and amino acid metabolism. The genes encoding RNA polymerase σ factors involved in different stages of *C. perfringens* sporulation were uniformly upregulated in the investigated isolates. The most prominent difference was observed for *cpe* gene expression. However, isolate 16SBCL940 showed increased transcription of *cpe* gene encoding CPE which was further identified as powerful CPE producer compared to the other four isolates included in this study. Similarly, genes encoding putative virulence factors (collagenase, hyaluronidase and phospholipase C) were downregulated. These downregulated genes are predicted to be expressed during vegetative growth of *C. perfringens* isolates.

Conclusions: These findings demonstrate that there are growth phase-specific differences in the global transcriptomes of CPE-positive *C. perfringens* isolates, and highlight the utility of comparative transcriptomics for identifying additional factors that are directly or indirectly involved in *C. perfringens* foodborne diseases. Reverse Transcriptase RT-PCR analysis on a set of selected functional and virulence factors gene expression profiles were will be needed to confirm this RNA-seq data.

[P002] EXPLORING THE EVOLUTIONARY SUCCESS OF THE ANTIBIOTIC-RESISTANT SALMONELLA KENTUCKY ST198

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Aim:
Salmonella enterica serovar Kentucky (*S.* Kentucky) is a common causative agent of gastroenteritis in humans. It is one of the most notorious Salmonella serotypes, as it is strongly associated with antimicrobial resistance. Ciprofloxacin-resistant S. Kentucky (CIPR S. Kentucky) belongs to a single sequence type (ST198), which recently acquired chromosomally encoded $bla_{CTX\text{-}M\text{-}14b}$ and spread across the EU continent, which was subject to urgent Inquiry (UI-464) of the ECDC. This PhD research elucidates this transposition from plasmid-borne to chromosomal resistance in single-cell resolution and investigates several factors that drive the resistance acquisition.

Methods:
We are developing fluorescent reporters based on two orthogonal *parS*/ParB systems to differentially label the plasmid backbone and the *ISEcp1-bla* $_{CTX\text{-}M\text{-}14b}$ unit. The reporter systems should enable studying the dynamics of conjugation and transposition at a cellular level using time-lapse fluorescent microscopy and microfluidics

Results:
In *Salmonella* spp., the resistance genes are largely encoded on mega-plasmids belonging to the incompatibility group H (IncH). Comparative genomic analysis of ESBL-producing *S*. Kentucky in Europe indicated a chromosomal integration of 2.5KB plasmid fragment harboring the *bla* $_{CTX\text{-}M\text{-}14b}$ adjacent to an insertion-like sequence- *ISEcp1*. *ISEcp1* can mobilize *bla* $_{CTX\text{-}M}$ gene from the plasmid to the chromosome and enhances its expression. During the first year of this PhD project (KENTUCKY) we cloned the transposition unit (TU) containing *ISEcp1-bla* $_{CTX\text{-}M\text{-}14b}$ into a prototype IncHI plasmid, R27. Next, We labeled the R27 backbone and TU with Phage-derived and *Lactococcus lactis*-derived *parS* sequences, respectively.

Conclusions:
The transposition of resistance genes from plasmid to chromosome adds an important dimension to the bacterial resistance, and since the cell biology of horizontal gene transfer dynamics has hardly been addressed, understanding the drivers of this transfer can help to control its occurrence.

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[P004] COLISTIN RESISTANCE IN SALMONELLA ENTERITIDIS ST11 IN THE POULTRY INDUSTRY INTERFACE, PORTUGAL 2014-2020

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Aim: *Salmonella* Enteritidis is the main serovar in reported human cases of gastrointestinal infection in the EU. Here, we describe the susceptibility profile of *S.* Enteritidis of animal origin of the last six years and characterized the genome of selected *S.* Enteritidis strains, by Whole Genome Sequencing (WGS).

Methods: One hundred and forty-six isolates of *S.*Enteritidis from poultry, meat and feed were submitted to antimicrobial susceptibility testing. Isolates with colistin MIC of ≥2µg/ml were evaluated by PCR targeting *mcr* gene (*mcr*-1 to *mcr*-9). Thirty-nine selected isolates were subjected to WGS by the Illumina NovaSeq platform. SPAdes and Prokka were used to *de novo* assembly and genome annotation. Genomic analyses were performed using the Center for Genomic Epidemiology tools.

Results: Overall, resistance to fluoroquinolones (23%) and colistin (13%) were the most prevalent. No *mcr* genes were found either in colistin resistant or susceptible isolates. Other mechanisms linked to mutations in chromosomal genes involved in the LPS modification are being studied. All sequenced strains belonged to ST11 and carried *aac(6')-Iaa* gene. Chromosomal mutations on *gyrA* (D87Y; S83F) were responsible for the decreased susceptibility to fluoroquinolones. The bla_{CMY-2} was harboured by the unique isolate resistant to 3rd generation cephalosporins. Several plasmid replicons were identified, of which IncFIB and IncFII are prevalent.

Conclusions: *S.* Enteritidis ST11 seems to be well established in Portugal in the poultry industry. Particularly worrisome are the levels of colistin resistance, given its current significance in clinical practice. Molecular mechanisms involved in colistin resistance are still to be determined.
This research was conducted within the ADONIS research project funded through the One Health European Joint Programme by the EU's Horizon-2020 Research and Innovation Programme (grant 773830) and supported by the FCT grant PTDC/CVT-CVT/28469/2017.

[P004] DYNAMICS OF EXTENDEN SPECTRUM BETALACTAMASE RESISTANCE GENE BLASHV-12 IN A LAYING HEN COMMERCIAL FARM

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Aim: To study the dynamics of antimicrobial resistance determinants in a commercial laying hen farm where antimicrobials where scarcely used (Moreno *et al*., 2019) using the extended spectrum betalactamase (ESBL) resistance gene bla_{SHV-12}.

Methods: Faecal samples of animals (from day-old chicks to laying hens) of fours batches were analysed. McConkey agar plates with cefotaxime (1 mg/L) were used for recovery of presumptive ESBL *Escherichia coli*. Isolates with different antimicrobial resistance profiles were characterized by whole genome sequencing and those in which the bla_{SHV-12} gene was detected are shown.

Results: *Bla* $_{SHV-12}$ gene was identified in *E. coli* isolates belonging to multilocus sequence type 155 in the second batch when pullets were sampled at week 15. The gene was found in an *IncI1-26* plasmid harboring also tet*(A)* gene and a class 1 integron carrying *aadA1*, *aadA2*, *sul3*, and *cmlA1* genes. Moreover, the isolates had an I*ncX1* plasmid, harboring *qnrS1* and bla_{TEM-1B} genes. The same isolate was detected at week 24 in the same batch moved to a laying house (a colistin treatment was administered at week 23) and again four months later in another batch of laying hens (at 23 weeks) housed in a different laying house of the same farm.

Conclusions: These results suggest the environmental persistence of the bla_{SHV-12} gene in this farm for at least six months carried by the same plasmid located in identical *E. coli* isolates.

References:
Moreno *et al*., Vet. Microbiol. 2019, 230, 211-214.

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[P005] EMERGENCE OF MULTIDRUG RESISTANT SALMONELLA INFANTIS ST32 IN THE POULTRY INDUSTRY, PORTUGAL 2016-2020

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Aim: *Salmonella* Infantis was the third most common serovar identified in human cases in the EU. We studied the susceptibility profile of *S.* Infantis isolates from the last five years and characterized the genome of selected isolates by Whole Genome Sequencing (WGS).

Methods: Antimicrobial susceptibility profile of 94 *S.* Infantis isolates collected from poultry (broilers, laying hens, meat) and zoo animals between 2016-2020, was determined by broth microdilution. Twenty-one selected isolates were subjected to WGS by the Illumina NovaSeq platform. SPAdes, Prokka, NCBI BLAST and tools from Center for the Genomic Epidemiology were used for genomic analyses.

Results: Overall, the highest frequencies of resistance were towards fluoroquinolones (45,7%), sulphamethoxazole (42,6%), tetracycline (36,2%) and trimethoprim (25,5%). Of notice, colistin resistance was 6.4%. All sequenced strains belonged to ST32, and the WGS predicted phenotype fully correlates with the multidrug resistant profile. A variety of resistance determinants (*tet1; sul1; sul3; dfrA8; dfrA14*; *mcr-1.1*; *aac(6')-Iaa; aadA1; floR*) and chromosomal mutations on *gyrA* (D87Y; S83F) and *parC* (T57S) were found. The $bla_{\text{CTX-M-65}}$ was found in one extended-spectrum beta-lactamase producer isolate. Several plasmid replicons were identified, of which IncFIB and IncX4 are prevalent. Several isolates contained pESI plasmid markers, namely K88 and *fim* genes.

Conclusions: Over the last years *S.*Infantis has become more prevalent in poultry flocks in Portugal, and a growing portion of strains are multidrug resistant, representing an increasing health hazard. Additional genomic studies are being done, to determine the presence of pESI-like plasmid, which could be associated with the dissemination of this serovar through the food chain.

This study was supported by the FCT grant PTDC/CVT-CVT/28469/2017

[P006] A SCOPING REVIEW ON THE IMPACT OF HEAVY METALS IN THE AGRI-FOOD ENVIRONMENT AS A SELECTIVE PRESSURE FOR ANTIMICROBIAL RESISTANCE

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Aim: Antimicrobial resistance is one of the greatest threats facing human and animal health worldwide. Heavy metals such as zinc and copper can be naturally present in the environment and are also commonly used as feed additives in animal production, as well as for disease treatment. Heavy metal resistance genes can be co-located or provide cross resistance to antimicrobials. The hypothesis is that this can act as a selective pressure for the selection and spread of antimicrobial resistance in the primary production and impact on transmission into the food chain. The objective of this study was to undertake a scoping review to map the literature on this topic and identify knowledge gaps.

Methods: A specific research question was defined, and a search string created using key words related to the research question. Three databases relevant to the research area were interrogated using the search string and articles identified that met specific criteria. These articles were screened, the relevant data extracted, and all findings combined to draw conclusions and identify knowledge gaps.

Results: This methodology supported the identification of key concepts and gaps in the knowledge regarding the impact of heavy metals on the development and dissemination of antimicrobial resistance in the environment and food chain. Variations in the methodologies used in studies make comparisons difficult.

Conclusions: The findings of this scoping review demonstrate the need to further investigate the impact of heavy metals as a selective pressure for shaping the resistome composition, particularly, in low and high metal containing areas and areas impacted by land spreading of animal waste.

Funding: This is co-funded by One Health EJP and Walsh Scholar Scheme Teagasc

[P007] DETECTION OF THREE SWINE-RELATED VIRUSES (HEPATITIS E VIRUS - HEV, MAMMALIAN ORTHOREOVIRUS - MRV, AND TORQUE TENO SUS VIRUS - TTSUV) IN THE WILD FAUNA OF NORTHERN ITALY

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Aim: This study evaluates the occurrence of Hepatitis E virus (HEV), Mammalian orthoreovirus (MRV), and Torque teno sus virus (TTSuV) in wild animals in Northern Italy to better estimate the risk of transmission to farmed animals and possibly to humans.

Methods: Liver samples were collected from wild boars (n=405), wild ruminants (red deer, roe deer, and chamois; n=128), and hares (n=165) in areas with different characteristics: mountains (Alps and Prealps) with low farm density and anthropization; and flatland (Po Valley) with high farm density and anthropization. Detection and identification of the pathogens were performed by PCR and sequencing.

Results: HEV detection was restricted to wild boars in the Po Valley (22.29% prevalence), while MRV was found in all studied species mainly in the mountains (up to 47.83%). MRV and TTSuV2 were found with high prevalence in hares living in both areas (49.09% and 37.58%, respectively). TTSuV1 was instead seldomly detected mainly in wild boars in the mountain areas (3.48%).

Conclusions: Our findings shed light on the distribution of three swine-related viruses in wild fauna in Italy. Given the zoonotic potential of HEV, wild boar surveillance in areas with intensive pig farming would be advisable. The high prevalence of MRV and TTV in hares suggests their role as reservoir and potentially in viral amplification: their surveillance could be helpful to monitor viral diffusion and risk for farmed animals. The joint surveillance of domestic and wild animals could enhance the safety of farmed animals and protect human health in a One Health perspective.

Funding: This research was funded by the Italian Ministry of Health, grant number RF-2016-02361926.

[P008] GAME MEAT OF CULINARY IMPORTANCE AS A POSSIBLE SOURCE OF HEPATITIS E VIRUS (HEV) INFECTION IN POLAND

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Aim: Assessment of the occurrence of hepatitis E virus (HEV) in wild boar and roe deer meat intended for human consumption.

Methods: In total, 116 muscle samples of culinary importance (neck, supraspinatus, intercostal, supraspinatus scapula and the longest back muscle) were collected from wild boar and roe dear. Virus extraction and isolation of viral nucleic acids were performed using TRIzol (TRI Reagent®) and a NucliSens kit (BioMérieux). For a quantitative detection of HEV RNA in meat samples, a real-time duplex RT-qPCR was employed followed by a subtype identification of the detected HEV strains. Moreover, the presence of anti-HEV antibodies in meat juice samples from game meat (44 samples) was assessed using an ID Screen® Hepatitis E Indirect Multi-species ELISA (IDvet).

Results: HEV RNA was solely detected in two out of 96 samples of intercostal muscles of wild boar at the low concentration $< 10^2$ virus G.C./g of muscle tissue. HEV was not detected in any out of 20 muscle samples of roe dear. The sequence analysis of the only one ORF2 genome fragment of the detected wild boar HEV strain revealed the presence of unidentified subtype clustered within gt3. The anti-HEV IgG were found in three samples of wild boar meat juice and none of the tested meat juice sample of roe dear contained virus-specific antibodies.

Conclusions: Wild boar meat can be a potential source of foodborne HEV infection for humans. The detected wild boar HEV strain belong to the zoonotic genotype 3.

[P009] EDIBLE PORK SLAUGHTER BY-PRODUCTS AS SPORADIC SOURCES OF ZOONOTIC HEPATITIS E VIRUS IN POLAND

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Aim: Evaluation of the occurrence of hepatitis E virus (HEV) in pig's blood and liver for human consumption.

Methods: In total, 246 samples of retail liver (n = 100) and pooled pig's blood (n = 146) collected from animals at slaughter were analysed for the presence of HEV. The virus genomic material, including RNA of a sample process control virus was isolated from food samples using a QIAamp® Viral RNA Mini Kit. Virus-specific IAC-controlled real-time PCR method was used for HEV detection.

Results: HEV RNA was found in 6 (2.4%; 95% CI: 0.9–5.2) out of 246 samples of tested foodstuffs. The virus was detected in pig's blood (3.4%; 95% CI: 1.1–7.8) and liver (1.0%; 95% CI: 0.0–5.0) with no significant differences observed in the frequency of its occurrence between tested food samples ($t = 1.33$; $p = 0.182 > 0.05$). The viral load in blood was ≤ 9.0 × 104 G.C./ml, whereas in liver it was as low as 16 G.C./g. The HEV strains belonged to the 3f and 3e subtype groups.

Conclusions: Although HEV was detected in pig's offal only sporadically, consumers cannot treat its occurrence with disregard as it demonstrates that HEV-contaminated pig tissues can enter the food chain.

[P010] EJP-OH-CARE: PILOT STUDIES TO SUPPORT GUIDANCE TO QUALITY ASSURANCE TESTING OF LABORATORY METHODS WITHIN A ONE HEALTH CROSS-SECTORAL FRAMEWORK

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Aim: EJP-OH-CARE aims at developing quality assurance or proficiency testing (PT) schemes to assess the combined performance of the OH sector. The work focus on three fields, detection/isolation, WGS based bacterial typing, and WGS based outbreak surveillance.

Methods: Three pilot PTs will be conducted in 2021.
A PT on detection/isolation will evaluate the laboratories capability to detect/characterize pathogens in relevant matrices that includes *Campylobacter*, *Salmonella* and *Yersinia*. Laboratories are requested to detect and characterize to species- (*Campylobacter*), serovar- (*Salmonella*) and biotype (*Yersinia*) using their routine methods.

A WGS based typing PT will assess the comparability of WGS data by evaluating quality metrics and proficiency in assignment of subtypes/attributes, e.g. sero- and sequence types, resistance, and toxins. Cultures and samples of DNA from *Campylobacter*, *Salmonella* and *Escherichia coli* will be included and both the individual and collective performance will be evaluated. The PT includes a benchmarking component where the PT results can be compared with outputs from other institutions bioinformatics pipelines.

A PT on outbreak surveillance will include WGS data from a distribution of 100 field isolates that encompasses outbreak clusters of *Campylobacter*, *Listeria monocytogenes* and *Salmonella*. The participants shall analyse the data using their current pipelines and report back those isolates which constitute an outbreak cluster. The outbreak exercise will take place in real-time over a period of two weeks.

Results: The pilot PT's are currently being undertaken and the results will be presented.

Conclusions: The experiences from the pilots will support the design of future cross-sectoral OH PT schemes.

[P011] ASSESSING CORONAVIRUS RISKS: FEASIBILITY OF PREDICTING TRAITS OF CLINICAL AND EPIDEMIOLOGICAL RELEVANCE FROM SEQUENCE DATA

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Aim: The TELE-Vir consortium aims to develop a toolkit for the real-time identification and characterization of emerging virus threats. One main objective is to provide a bioinformatics environment where genetic data can be analysed, and integrated with phenotypic and epidemiological data to enhance risk assessment and surveillance. Focusing on coronaviruses, we carried out a scoping review and expert elicitation (i) to identify phenotypes of importance in assessing a virus' potential as a health threat; and (ii) to assess the feasibility of their prediction from genetic data.

Methods: We searched the literature for coronavirus phenotypes of epidemiological or clinical relevance, their genetic determinants, and any previous predictions on the basis of genetic data. For each phenotype, the feasibility of implementing its prediction was assessed based on the strength of the association; quantity and quality of available data; and technical feasibility. Additionally, a survey was circulated to partner institutes and associated virologists to elicit their opinion on phenotype prediction activities that would be valued in a genomic surveillance toolkit.

Results/Conclusions: The literature review and expert elicitation indicated that receptor usage, receptor-binding affinity and antigenic variation are among the most important phenotypes to predict for risk assessment of coronaviruses. Availability of structural data may allow some prediction of receptor binding, depending on virus similarity and structural modelling. The prediction of antigenic variation is reliant on integrating large serological datasets with genetic data, but such data are rapidly expanding for SARS-CoV-2. Work is ongoing to implement selected genotype-phenotype associations into the TELE-Vir bioinformatics toolkit.

Funding: This project is funded by One Health EJP

[P012] THE HARMONISED ONE HEALTH EJP DATA MANAGEMENT APPROACH ENSURES THAT THE DATA ARE FAIR (FINDABLE, ACCESSIBLE, INTEROPERABLE AND REUSABLE)

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Aim: One Health EJP (OHEJP) is funded under Horizon 2020. It is required that all data generated are FAIR: Findable, Accessible, Interoperable, Reusable. Data Management Plan (DMP) is a living document gathering information on project-generated (meta-) data. OHEJP data management aims for FAIRness in a One Health setting.

Methods: To harmonize the process of DMPs in the context of OHEJP, the DMP Committee investigated the available options. Four types of DMP template were identified and evaluated for suitability: a text file, a spreadsheet, a website solution and a web application for collaborative data management platform (CDP), all fulfilling Horizon 2020 FAIR data requirements.

Results: The CDP was selected as the template. The level of detail to be included was defined and harmonized with the overarching DMP for the OHEJP. The CDP was introduced to the projects running under OHEJP. By end of 2020, 16 project-level-DMPs were drafted on the CDP. The entries have relevant metadata, including keywords from the OHEJP-ORION glossary. The specific data can be made publicly available during the project lifetime, and finally, a file containing all DMP (meta-)data will be made public. To aid dissemination, linking the DMPs with the One Health Outcome Inventory (OHOI) is planned.

Conclusions: For a large project like OHEJP, harmonisation of data management is crucial. A harmonised data management approach ensures that the data are FAIR in a One Health setting.

[P013] COMPARATIVE STUDY OF BACTERIAL DNA EXTRACTION METHODS ON COMPLEX SAMPLES FROM THE MARINE ENVIRONMENT.

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Aim: Extraction of high quality DNA is a critical step for successful molecular analysis, such as qPCR (quantitative polymerase chain reaction) or metagenomics. Nevertheless, extracting bacterial DNA from marine environment samples can be a challenge, especially due to the presence of inhibitors. The aim of this study was to compare 7 extraction methods on marine samples (plankton, bivalves and fish) to collect quality bacterial DNA amplifiable by qPCR.

Methods: We extracted bacterial DNA from phytoplankton, zooplankton, bivalve mollusk flesh, fish skin, gill and gut samples with 6 commercial kits: DNeasy Blood and Tissue, PowerBiofilm, PowerSoil kits (Qiagen), GenElute Stool (Sigma), PureLink Microbiome (Invitrogen), Wizard Genomic (Promega) and one thermal shock lysis method. The purity and quantity of extracted bacterial DNA were determined by spectrophotometry. Moreover, the amplifiable bacterial DNA was quantified by qPCR targeting *tuf* and *rpoB* genes (two bacterial housekeeping genes) and *hlyA* gene encoding Listeriolysin O protein that it was a specific to the internal control *Listeria monocytogenes*.

Results: The highest DNA concentration, with acceptable purity, was obtained using the PowerBiofilm kit. The qPCR data clearly showed that the PowerBiofilm and PureLink Microbiome kits were the most optimal for extracting amplifiable bacterial DNA.

Conclusions: The PowerBiofilm kit has been showed to be the most suitable, allowing the extraction of good quality, quantity and amplifiable bacterial DNA of different nature marine samples. It was important to have an appropriate DNA extraction method to the future study of metagenomic in antibiotic resistance at different marine trophic levels.

Keywords: DNA extraction, marine microbial ecology, RT-PCR.

[P014] DETERMINATION OF SELECTION PRESSURES IN ENVIRONMENTAL SAMPLES

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Aim: The project FED-AMR (The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain) consists of 6 workpackages. The task of workpackage 4 ist the determination of selection pressures in environmental ecosystems caused by antimicrobials, elements and herbicides.

Methods: The following substances are in the analytical scope:
8 elements (heavy metals): Cd, Co, Cr, Cu, Hg, Ni, Pb, Zn analysed by ICP/MS
7 herbicides including degradation products: glyphosate / AMPA, glufosinate, 2,4-D, metolachlor (incl. ESA and OA), bentazon and metazachlor (incl. ESA and OA) analysed by LC/MSMS
29 antimicrobials (4 tetracyclines, 7 sulfonamides, 7 macrolides, 10 fluoroquinolones and trimethoprim) analysed by LC/MSMS

As yet more than 100 samples from different environmental compartments (soil, water, manure, plants, feed) were collected.

Results:
Preliminary results (maximum values in brackets):
Elements were quantified far below legal threshold values. Herbicides were found in soil, water and manure samples: glyphosate (42 µg/kg), AMPA (170 µg/kg), glufosinate (10 µg/kg), metolachlor ESA (18 µg/kg). It is not likely that these low concentrations of elements and herbicides perturb bacterial populations.

Antimicrobials were not detected in all 36 soil samples, azithromycin (0,29 µg/L) in 4 water samples, doxycycline (98 µg/kg) and marbofloxacin (18 µg/kg) in 3 manure samples. These concentrations of doxycycline and marbofloxacin exceed the minimum selective concentration by a factor of 6,5 and 180 respectively.

Conclusions:
It is likely that the detected antimicrobials in manure can select resistant bacteria. The samples will be analysed, if there are higher concentrations of antimicrobial resistance genes.

[P015] EUROPANELOH: CONSTRUCTION OF A CARE CATALOG OF BACTERIA FOODBORNE PATHOGENS STRAINS USED AS REFERENCE MATERIALS IN THE EUROPEAN UNION

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Aim: Microbiological Reference materials (RM) are microbial strains, considered as standard that can be used to verify the quality and accuracy of methods in the microbiology field. To be considered as standard, RM must meet a set of quality criteria, associated metadata, phenotypic, and genomic information. They are greatly needed in clinical, food and veterinary laboratories for quality control, validation of new methods, as standards in proficiency testing (PT) trials and globally for the assessment of the quality and reliability of the data produced in food safety and public health sectors. The work presented here is part of the activities of CARE (Cross-sectoral framework for quality Assurance Resources for countries in the European Union) joint integrative project of the OH EJP. The aim is to create a catalog of perfectly characterized bacterial strains (and associated genomes), named "EUROpanelOH", that will be accessible through a CARE web portal.

Methods: The first step to build the EUROpanelOH was to define a list of nine prioritized zoonotic and pathogenic bacterial species: *Salmonella, Listeria monocytogenes, Campylobacter, Diarrheagenic E. coli, Staphylococcus aureus, Streptococcus, Bacillus cereus, Vibrio,* and *Yersinia enterocolitica*. Based on this list, an inventory of RM available among the members of the CARE Consortium was obtained, and a preliminary database was built with unique identification for each strain including information about the relevance for being considered as RM. Each RM is associated with metadata related to the isolation, growth characteristics, phenotypic and genetic information such as virulence or toxic genes, genomic information, MALDI-TOF spectra, AMR resistance profiles and information about previous use as standard or in PTs.

Results: A total of 2719 bacterial strains from 12 Institutes were collected from Human, Animal, Food, and environmental sectors for 13%, 62%, 11% and 6% respectively. Most of the isolates were correctly defined according to requested metadata, around 25% of them were already whole genome sequenced, nevertheless, phenotypic and genotypic on antimicrobial resistance data often were missing and will be completed furthermore. The next step will be to consolidate all the above mentioned data of this first RM panel into a sustainable and scalable database, that will constitute the EUROpanelOH. This collection will be maintained, stored and made accessible under certified quality standards, particularly in Biological Reference Centres (BRC). In addition, a searchable web portal will make the RM collection visible and accessible and will provide functionalities such as contacts of all strain distributors, the indexing of all forms and permits needed to order strains from different BRCs.

Conclusions: Implementation of EUROpanelOH results from harmonized efforts made by different European laboratories with deep technical and biological expertise. The CARE catalog of foodborne bacterial pathogens RM is an original initiative in the field of microbiological food-safety that will provide an efficient benchmarking tool useful for rapid and accurate referencing in cross-sectoral microbiological analysis, PTs implementation as well as for international collaborations.
Project CARE is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

[P016] DEVELOPMENT OF AN APTAMER-BASED TEST FOR TRICHINELLA DETECTION

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Aim: Trichinellosis is a zoonotic illness transmitted through the consumption of raw or undercooked meat products infected with the parasitic nematode *Trichinella* spp. Excluding its brief existence as a free-living migratory larva, *Trichinella* spp. resides primarily within the confines of its host's muscle cells. In this study, we employ an innovative and novel larval-based selection method to produce a set of *Trichinella spiralis* specific aptamers for use in an accurate diagnostic method for animal and human infection.

Methods: A magnetic stirrer method of artificial digestion and microscopy were used for the recovery and quantification of *Trichinella spiralis* muscle larvae (ML). Aptamers were selected from a synthetic single-stranded DNA (ssDNA) library composed of 10^{13}-10^{16} random sequences by an iterative *in vitro* selection process termed Systematic Evolution of Ligands by EXponential enrichment (SELEX). Additionally, the selection process and sequence pool quality were monitored by gel electrophoresis.

Results: Currently, *T. spiralis* ML have been successfully isolated from experimentally infected mice and preserved in 70 % ethanol. Furthermore, 5 rounds of SELEX have been successfully performed based on gel electrophoresis results, indicating the presence of high quality aptamer sequences.

Conclusions: In light of these results, additional cycles of selection are to be performed while applying new methods to obtain high quality aptamer sequences. Furthermore, melting curve analysis and confocal microscopy will be implemented to more accurately evaluate aptamer pool diversity and specificity. Finally, *T. spiralis* specific aptamers could act as promising candidates as molecular recognition elements in diagnostic assays or for therapeutic purposes.

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[P017] CAN THE AGRICULTURAL MICROBIOME BE UTILISED FOR SUPPRESSING ZOONOTIC PATHOGENS IN PLANT FOODS?

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Aim: Plant based foods are a significant contributor to foodborne disease outbreaks. Whilst human pathogen numbers in the horticultural production environment generally reduce over time, there may be residual presence, resulting in a potential food safety risk. There is extensive knowledge of the different risk factors in horticultural production, but less is known about the interactions between human pathogens and the microbial communities of the plants, and whether the latter can exert a suppressive effect on the former. The objective of this work is to present some perspectives on harnessing agricultural microbiomes towards increased antagonism against zoonotic pathogens in horticultural production systems.

Methods: Published studies were reviewed for evidence of suppressive interactions between pathogens and native microbial communities in different ecological niches in plant production systems.

Results:

- Suppressiveness is likely to be a function of the community as a whole
- Conditions facilitating expression of suppressiveness need to be elucidated
- Multidisciplinary approaches including relevant knowledge on plant physiology, agronomy, agricultural microbiota and the food chain are needed to develop strategies to investigate functional interactions
- Combining genomic approaches, network analysis, dynamic monitoring and ecological modelling will enable better understanding of the functional potential, ecological niches occupied, and how microbes interact with each other and the plant.

Conclusions: As our understanding of the factors shaping microbial assembly, plant-microbe interactions and microbial function in plant systems is enhanced, utilisation of the agricultural microbiome for suppressing zoonotic pathogens seems a realistic prospect. This will be of significant importance from a food safety point of view.

[P018] INTRANASAL INFECTION OF FERRETS WITH SARS-COV-2 AS A MODEL FOR HUMAN AND MINK INFECTION

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Aim: Characterisation of severe acute respiratory syndrome (SARS)-related coronavirus 2 (SARS-CoV-2) infection dynamics and host response in the ferret model.

Methods: Ferrets were experimentally inoculated with SARS-CoV-2 and sampled longitudinally to monitor viral RNA and antibody levels. Viral RNA and protein levels were evaluated in tissues at necropsy. Environmental levels of viral RNA were also assessed.

Results: Intranasal inoculation resulted in subclinical infection and viral RNA quantified between 2 and 21 days post-inoculation (dpi) peaked in the upper respiratory cavity between 4 and 6dpi. Viral RNA and protein were detected primarily in upper respiratory tract (URT) tissues, notably in cells of the respiratory and olfactory mucosae of the nasal turbinates, including olfactory neuronal cells. Antibody responses to the spike and nucleoprotein were detected from 21dpi, but virus-neutralizing activity was low. Re-exposure of two ferrets by repeat virus inoculation after a 17-day interval did not produce viral RNA shedding, but did amplify the humoral response in one animal. Viral RNA was not detected in environmental samples of food, water or swabs of metal cage surfaces but was detected in swabs of ferret fur. SARS-CoV-2 genomic sequence analysis in URT samples from three animals identified a nucleotide substitution, compared to the inoculum, that corresponded to the spike protein Y453F variant. This same substitution was also reported in the ‘Cluster 5’ variants identified in mink from Denmark.

Conclusions: Ferrets can be experimentally infected with SARS-CoV-2 and are a suitable species for assessing virus-host interactions in mink and for modelling human asymptomatic infection.

[P019] DISSEMINATION OF ANTIMICROBIAL RESISTANT CLOSTRIDIUM DIFFICILE RT078/ ST11 IN AUSTRIA ACROSS THE HUMAN-ANIMAL-WILDLIFE INTERFACE

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Aim: *C. difficile* strains resistant to antibiotics including RT078/ST11 appear to be successfully disseminated thanks to selective pressure. We aimed at assessing the genetic relatedness, genetic and phenotypic resistances of *C. difficile* isolates obtained from environmental, animal and human sources in order to monitor the dissemination of resistant RT078/ST11 *C. difficile* across the human-animal-wildlife interface.

Methods: Within the FED-AMR project, we collected in 2020 85 samples from interconnected environmental compartments in an open air laboratory in Austria. The obtained isolates after sample cultivation were used for Whole Genome Sequenced-based typing and testing of *C. difficile* toxin production. AMR was confirmed by E-test for clindamycin, metronidazole, rifampicin, vancomycin and moxifloxacin. RT078/ST11 isolates were compared with 20 human and two pig RT078/ST11 isolates collected between 2016 and 2019.

Results: We recovered seven toxinogenic *C. difficile* RT078/ST11 isolates (n=3, manure; n=3, wastewater; wildlife, n=1) from the 85 samples. Five isolates were phenotypically resistant to moxifloxacin and one to clindamycin. All were susceptible to vancomycin, metronidazole and rifampicin. The *cde*A (n=6), *tet*40 (n=3), and *efp*A (n=1) resistance genes coding for efflux pumps were detected. Four of the moxifloxacin resistant isolates obtained from manure, wastewater and wildlife samples, including the clindamycin resistant isolate, clustered together by cgMLST with four human isolates from 2018 and 2019 (cluster threshold ≤6 alleles).

Conclusions: cgMLST revealed a cluster of resistant RT78/ST11 *C. difficile* isolates from human, animal and environmental sources, and therefore confirming its zoonotic traits. Continuous monitoring of resistant RT078/ST11 *C. difficile* under a One Health umbrella is needed to prevent its further dissemination and outbreaks.

Funding: This project is funded by the One Health EJP

[P020] A BRIEF EXCURSION INTO PARADISE

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Aim: The PARADISE project primarily focuses on the zoonotic pathogens *Cryptosporidium parvum* and *Giardia duodenalis*, which can cause diarrhoeal disease in humans and animals, worldwide, and have been associated with food- and water-borne outbreaks in Europe and elsewhere. The main aims include the development of new tools for the genetic characterization of isolates and new strategies for enrichment of these pathogens from complex matrices.

Methods: The project is organized into three research-oriented work packages. WP2 activities are focusing on NGS-based genomics and metagenomics, WP3 focuses on development and validation of new molecular typing schemes, and WP4 explores the use of nanobodies, aptamers and hybridization probes for new enrichment strategies.

Results: The generation of many new *Cryptosporidium parvum* and *Giardia duodenalis* whole genomes has allowed a rationale design of novel typing schemes with improved resolution. Detection of foodborne parasites in complex matrices is being explored using amplicon-based and shotgun metagenomics, as well as with protocols to capture parasite-specific DNA sequences. Nanobodies and aptamer technologies are being optimized to design novel enrichment protocols.

Conclusions: This project will place Europe at the forefront in the fields of comparative genomics and metagenomics and will have a large impact on the molecular epidemiology and the detection of parasites/parasitic DNA in complex matrices in a one health setting (human, animal, environment, food).

Funding: The PARADISE project is supported by funding from the European Union's Horizon 2020 Research and Innovation Programme, under grant agreement No 773830: One Health European Joint Programme.

[P021] COMPARATIVE GENOMICS OF CRYPTOSPORIDIUM PARVUM AND GIARDIA DUODENALIS ISOLATES FROM EUROPE: TOWARDS THE DEVELOPMENT OF NOVEL GENOTYPING SCHEMES

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Aim: The protozoa *Cryptosporidium parvum* and *Giardia duodenalis* are causes of diarrhoeal disease in humans and animals. Typing schemes available to differentiate between isolates often lack resolution, limiting infection source tracing and outbreak characterisation. To develop novel typing schemes, the PARADISE project is generating whole genome sequencing (WGS) data for identification of loci with high genetic variability.

Methods: Isolates of *C. parvum* (n=55) and *G. duodenalis* assemblage B (n=25) were collected from across Europe, and processed to extract high-quality genomic DNA for WGS (Illumina). For identification of variable regions, two bioinformatics pipelines were established. Common criteria to select variable regions included the number of Single Nucleotide Polymorphisms (SNPs), their distribution on chromosomes, and their relative usefulness in distinguishing between isolates within a population.

Results: High quality genome drafts were obtained for almost all isolates. Raw sequence data processing identified about 100 highly variable genomic regions within *C. parvum* and *G. duodenalis* assemblage B.

Conclusions: The large dataset of WGS data has enabled better evaluation of the overall genetic variability in *C. parvum* and *G. duodenalis* at the European level. The loci identified with high genetic variability are being evaluated for inclusion in novel genotyping schemes. This should improve both source attribution and knowledge of the molecular epidemiology of these zoonotic pathogens.

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[P022] LIGHTS AND SHADES IN GENOTYPING: EUROPEAN TOXOPLASMA GONDII NEEDS A CLOSER LOOK USING HARMONISED APPROACHES

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Aim: Despite many efforts on *Toxoplasma gondii* genetic characterization, an entirely clear picture of the population structure in Europe has not been achieved, yet. The present study aimed to summarize the available information and provide a map of the genotype distribution of the circulating strains.

Methods: Despite many efforts on *Toxoplasma gondii* genetic characterization, an entirely clear picture of the population structure in Europe has not been achieved, yet. The present study aimed to summarize the available information and provide a map of the genotype distribution of the circulating strains.

Results: Among 114 European genotyping studies found, only 50 implemented at least four PCR-RFLP or PCR-sequencing markers, involving 801 samples from humans, domestic or wild animals, or environment. Among all specimens analyzed, 79.5% corresponded with type II, 8.1% with type III, 1.6% with type I, and finally, recombinant or atypical strains, and mixed infections accounted for 10.7% of the records. In contrast, 19 studies using microsatellite(MS)-typing (15 MS markers, involving 490 samples from humans, livestock, wildlife and pet animals) revealed 91.0% strains of type II, 4.5% of type III and 1.6% of type I. In addition, MS-typing indicated atypical, mixed, or imported strains in 2.9% of the samples. There is a lack of consensus over the methodologies and markers applied. Sampling disparities exist between regions, and vast areas remain unexplored; scarce data is available particularly from human cases.

Conclusions: There is consensus on type II *T. gondii* prevailing in Europe, but the absence of normalization in the use of typing methods limits detailed knowledge. Standardized, high-end typing tools and integrative strategies are needed to fill the gaps and provide a clear picture of the *T. gondii* population in Europe.

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[P023] FASCIOLA SPECIES INTROGRESSION: JUST A FLUKE OR SOMETHING MORE?

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Aim: To highlight the importance of hybridization and introgression between *Fasciola* spp. and the role of the international trade of livestock in facilitating parasite translocation events.

Methods: The threats posed by a range of viral and bacterial zoonotic diseases inevitably receive renewed attention in the wake of global pandemic events due to their overt and devastating impacts on human health and the economy. Chronic and subclinical diseases, however, such as those caused by infection with parasites, are largely overlooked despite affecting millions of people each day. Parasitic diseases of livestock are of particular concern due to the additional threat they pose to animal productivity and food security. In the case of fasciolosis, caused by infection with *Fasciola hepatica* or *Fasciola gigantica*, this oversight has allowed the expansion of areas of parasite sympatry in association with the international trade of livestock and thus increased incidences of hybridisation and possible introgression between the two species.

Results: The terms 'hybridisation' and 'introgression' are frequently used without differentiation throughout the *Fasciola* spp. literature and often without any consideration of the functional and epidemiological implications of either state. Introgression between these two species, if it exists, may have important human and animal health consequences, including increased drug resistance, infectivity, virulence and pathophysiology.

Conclusions: Here we highlight how an increased demand for animal-derived protein, combined with a lack of appropriate tools for detection of these events, is changing the *status quo* of these zoonotic parasites.

[P024] A SEMI-AUTOMATED IN VITRO MODEL TO STUDY THE TRANSMISSION DYNAMICS OF ANTIMICROBIAL RESISTANCE IN THE CHICKEN CAECAL MICROBIAL COMMUNITIES

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Aim: The continuous surveillance of antimicrobial resistance (AMR) in farm animals is critical to safeguarding public health. Alternative methods like *in vitro* gut models provide valuable insight and knowledge on the AMR transmission dynamics within and between microbial communities without using live animal studies. Understanding these microbial dynamics is essential for developing interventions that can reduce AMR spread among chickens and between chickens and humans. In this research, we aimed to: a) establish a semi-automated *in vitro* system able to mimic the physiological conditions of the chicken caeca and maintain the main microbial communities, and b) study the dynamics of the *in vitro* cultured caecal microbiota over time.

Methods: Chicken caecal physiological conditions (pH, temperature, caecal movements and anaerobic environment) were simulated, and real-time monitored in a continuous single-stage fermentation culture system (Applikon®) for nine days. Two systems were employed, each with a working volume of 100 ml (90 ml of Viandre-Leuvre (VL) media and 10 ml of a pre-reduced and preserved pool of chicken caecal inoculum). *In vitro* cultured microbiota was fed with VL media at a constant rate. Samples from both systems were daily collected for culture-dependent and independent (16S rRNA gene) analyses

Results: pH, temperature, and anaerobic conditions remained stable in both systems until the end of the process. Culture-dependent and microbial composition analyses (16S rRNA-gene analysis) are currently ongoing.

Conclusions: Our preliminary results suggest that a semi-automated *in vitro* chicken caecal system can mimic and maintain the physiological conditions needed to study AMR's transmission dynamics in chicken caecal microbial communities.

Funding: This study is funded by One Health EJP

[P025] DIFFERENCES IN CAECAL MICROBIOTA COMPOSITION OF BROILER CHICKENS COLONISED AND NON-COLONISED WITH ESBL-ESCHERICHIA COLI

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Aim: *Escherichia coli* strains expressing Extended-Spectrum Beta-Lactamases (ESBLs-Ec) have emerged globally in livestock with a high prevalence in poultry production. The chicken's caeca harbour complex and dynamic microbial communities. Among them, *E. coli,* a ubiquitous early coloniser and a potential reservoir for ESBLs-plasmid dissemination. This study aims to understand the successional dynamics of the chicken caecal microbiota of ESBL-colonised and non-colonised chickens and identify any existing difference therein.

Methods: 216 caecal samples from commercial broiler chickens were collected from day 0 to 35 after hatching, daily for the first week of life and weekly thereafter. Caecal samples were screened for ESBLs-Ec by selective culture. One hundred thirty-seven samples were sequenced (16S rRNA genes), targeting the V3-V4 region. Microbiota and statistical analyses were performed using R 3.6.1 and the DADA2, Phyloseq and Vegan packages.

Results: ESBLs-Ec was detected from day 2 in caecal samples with an increasing prevalence from 0.11, 95% CI [0.01; 0.34] to 1.00, 95% CI [0.81; 1.00] on day 35. Microbiota analysis revealed differences in bacterial community composition of ESBLs-colonised and non-colonised broilers. Chicken's age and ESBLs-Ec colonisation explained 15% of the caecal microbiota variation ($P<0.001$). No difference in microbial richness was found between ESBLs-colonised and non-colonised broilers.

Conclusions: Further studies will aim to elucidate the relation between the chicken caecal microbial composition and ESBLs-Ec colonisation.

Funding: This study is funded by One Health EJP

[P026] MEME PROJECT UPDATE ON ECHINOCOCCUS MULTILOCULARIS AND ECHINOCOCCUS GRANULOSUS S.L. IN EUROPE

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Aim: MEME is an international multicentre collaborative project that started in January 2020 and aims to fill research gaps highlighted by international agencies for the detection and control of cystic and alveolar echinococcosis in animal hosts.

Methods: MEME focuses on standardization, harmonization and validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools to detect *Echinococcus multilocularis* (Em) and *Echinococcus granulosus s.l.* (Eg) in the food chain. Production of epidemiological data on the presence of Em/Eg eggs in the food chain will focus on vegetables for human consumption and on canine faeces in selected endemic countries.

Results: During the first year of the project, MEME generated the key protocols and standard operating procedures to feed the main tasks in the project. Results on detections methods have already been published: 1) Comparison of two DNA extraction methods and two PCRs for the detection of Em in stool samples; 2) Bayesian Analysis of three methods for diagnosis of CE in sheep; 3) Microsatellite investigations of Eg cysts; 4) Species detection of Eg by novel probe-based real-time PCRs; 5) Validated method based on PCR-RFLP and multiplex PCR assay for the identification of Eg species; 6) Identification of Eg G1/G3 by SNPs assays (see https://onehealthejp.eu/jrp-meme/). The project is now collecting biological samples for producing epidemiological evidence on the presence of these parasites in different matrices.

Conclusions: MEME will provide a comprehensive set of integrative activities to harmonize procedures, improve detection of Eg/Em, and define control strategies.

Funding: MEME project is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

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[P027] OCCURRENCE OF TICK-BORNE-ENCEPHALITIS IN THE AUTONOMOUS PROVINCE OF BOLZANO – ITALY: AN EXAMPLE OF ONE HEALTH APPROACH IMPLEMENTATION AT LOCAL LEVEL

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Aim: In the Autonomous Province of Bolzano (Italy), the first tick-borne-encephalitis (TBE) human case was confirmed in 2000 and during the last decade the number of case reports has increased. Currently, even if the neighboring countries Austria and Switzerland are considered endemic for the disease, only an area southwest of the provincial capital Bolzano appears to be at risk. The main routes of TBEV transmission are tick bites and the consumption of nonpasteurized milk and since the safest preventative measure against TBE is active immunization, the Province offers free vaccination to residents of the affected area and to occupationally exposed workers.
Susceptible animals are usually asymptomatic carriers; however, some domestic species can be considered indicators for human TBEV infection risk. To clarify the epidemiological situation data concerning the occurrence of TBE in the Province, a study was conducted in 2018-2019 including dogs, horses and dairy goats as sentinel animals and humans. Moreover, samples of goat milk were tested.

Methods: A total of 600 samples from blood donors and representative samples of dogs and horses were tested for anti-TBEV antibodies. Moreover, goat milk samples were testes for the detection of anti-TBEV antibodies and viral RNA.

Results: Respectively 1,1% of donors, 3,3% of dogs and 3,4% of horses tested positive. The presence of anti-TBEV antibodies and viral RNA was not confirmed in goat milk

Conclusions: Although this study indicates a low prevalence of TBE, it highlights the importance of the One Health approach to monitor this emerging zoonosis at local level

[P028] ENABLING THE BROAD IMPLEMENTATION OF FULL-LENGTH SEQUENCING FOR IMPROVED SURVEILLANCE OF ANTIMICROBIAL RESISTANCE

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Aim: Current European monitoring systems for antimicrobial resistance (AMR) fall short in identifying the drivers of horizontal transmission of AMR genes. Although an increasing emphasis is put on genomic-based surveillance, mobile genetic elements (MGEs) remain challenging to reconstitute from short-read sequence data due to their chimeric, modular and repetitive nature.

Methods: The goal of the FULL_FORCE consortium will broadly introduce long-read sequencing in EU veterinary and public health institutes, by supplying 17 EU partners with a technological toolbox and hands-on training in MinION™ sequencing. This know-how will be applied on five study cases, and applications in metagenomics and AMR transmission models.

Results: The Full Force Plasmid Assembler (FFPA v1.0) was created. This python script joins best-in-field tools to trim and QC short and long sequence reads (qcat, Trimmometric), enables species identification through Kraken and performs either Nanopore or hybrid assemblies through Unicycler. The wet and dry lab performance of each partner is being assessed in a proficiency test, which includes full-length sequencing of five multi-drug resistant *Escherichia coli* isolates. Moreover, the Full_Force methodology is applied in the phylogenetic analysis of IncI1, IncK plasmids in cross-sectional datasets, of epidemic ESBL plasmids from horses across Europe, in the diversity of the pESI megaplasmid in EU-wide isolated *Salmonella Infantis* strains, and in the plasmidome of *Klebsiella pneumoniae* strains isolated in a One Health setting.

Conclusions: The effective implementation of long-read sequencing across public health and veterinary labs can provide a paradigm shift in EU AMR surveillance, delivering insight in dominant MGEs, which are driving resistance among commensal and pathogenic Enterobacterales in Europe.

Funding: This study is funded by One Health EJP

[P029] IN SILICO STUDY OF IS6110 SEQUENCES ABUNDANCE AND LOCALISATION EVOLUTION IN THREE MAIN MYCOBACTERIUM BOVIS FRENCH GENOTYPES OF ENDEMIC REGIONS

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Aim: Bovine Tuberculosis is a zoonotic disease caused by *Mycobacterium bovis*. IS*6110* is an insertion sequence of the *Mycobacterium tuberculosis* complex –to which *Mycobacterium bovis* belongs-, with a role in genome plasticity and in bacterium evolution. Although *Mycobacterium bovis* is described as possessing one or very few copies of IS*6110* (1), we showed that a third of French *Mycobacterium bovis* strains are IS*6110* multi-copy, 7% with more than 8 copies. Furthermore, strains with the highest IS*6110* numbers are currently circulating in bTB-prevalent regions. IS*6110* abundance also correlates with phylogenetic groups. A focus on strains of three endemic genotypes was carried out.

Methods: The copy number and localization of IS*6110* was sought in three major endemic groups: group 1, composed of 92 strains from Côte d'Or (2009-2014), group 2, 226 strains from Dordogne Haute-Vienne (2001-2017) and group 3, 187 strains from Pyrénées-Atlantiques (2002-2017). ISMapper (2) on Illumina reads was used for *in silico* studies with Mb3601 as the reference genome(3).

Results: A strong stability of IS*6110* copy number and a high recurrence of their genomic position over time was found. Analysis of IS*6110* position reveals disruption of some important genes involved in virulence, lipid metabolism and resistance of environmental stress.

Conclusions: In France, the proliferation and geographic expansion of strains of the three endemic groups in the last years is not accompanied by a modification of their genomes through an IS*6110* transposition active mechanism. Genetic modifications introduced by IS*6110* transposition could explain strong fitness on strains of endemic genomic groups.

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[P030] NEW REFERENCE GENOMES OF MYCOBACTERIUM BOVIS ADAPTED A FRENCH GENOTYPE DIVERSITY

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Aim: Bovine Tuberculosis (bTB) is a zoonotic disease due to *Mycobacterium bovis* (*M. bovis*). France has a bTB-free status but the disease has not been eradicated and a worryingly steady increase of bTB has been observed in some regions. This could be explained by the detection of bTB in wildlife that spills it back to livestock in the same territories. The transmission link between infected animals remains difficult to establish given that they share the same *M. bovis* genotypes. Whole genome SNP (single nucleotide polymorphisms) compared to appropriate reference genomes can precisely differentiate strains. However, new reference genomes genetically close to French field strains are required to perform these studies.

Methods: Ten strains representing each *M. bovis* French clonal group were selected. *De novo* sequencing with MinION and Illumina technologies was performed. The genomic synteny and pangenome analysis were carried out using Mauve and Roary tools. (Core Gene Phylogeny, Recombination events detection...).

Results: Ten new complete genomes of French *M. bovis* strains were obtained. The comparison of these complete genomes ascertained that the genome organization among them is remarkably stable. The detection of sequence polymorphisms, insertion sequences and deletions made it possible to specify the genomic traits characteristic of each clonal group.

Conclusions: Obtaining these new reference genomes allows us to better describe French clonal groups. They will be useful to better understand the dynamics of bTB between cattle and wild animals and to implement more effective control measures to eradicate bTB in these areas.

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[P031] MAPPING THE SURVEILLANCE ACTIVITIES FOR FOOD-HAZARDS ACROSS EUROPE

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Aim: MATRIX is part of the One Health European Joint Programme aiming at the implementation of One Health Surveillance (OHS), through the strengthening of the whole process of surveillance, and the implementation of guidelines for OHS improvement. The Project relies on a problem-based approach and focuses on four hazard-tracks: Salmonella, Listeria, Campylobacter and an emerging threat. As Work Package 2, a mapping of the surveillance chain across all sectors for each hazard-track has been performed from late 2020 to early 2021.

Methods: Per each hazard-track, including Hepatitis E selected as the emerging pathogen of interest, a specific food chain was selected to explore in detail the different realities. Twelve online questionnaires were implemented based on the ”farm to fork” approach for the three sectors (animal health, food safety and public health) and each hazard-food chain combination. Surveillance activities in place were investigated in at least two countries for each combination.

Results: Salmonella was investigated in humans and pork meat food chain; Listeria in humans and dairy products; Campylobacter in humans and poultry meat; Hepatitis E, in humans and wild boar meat. Answers were categorized in order to be graphically displayed, in: events, actors, data, metadata, event producing data, identified data sources, and sharing potential. Answers of these categories represent the start to identificate cross-sectorial linkages across surveillance chains.

Conclusions: The mapping of surveillance activities in place was the first step in the identification of best-practices for multi-sectorial collaboration. The following phase is the identification of outputs that could be shared for One Health oriented decision-making.

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[P032] CONCEPTUAL MODEL OF FACTORS IMPACTING GENOMIC CLUSTERING

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Aim: In order to provide a basis for a better informed integration of whole-genome-sequencing in epidemiological surveillance, we attempt to formalize the cluster detection problem. We propose a conceptual model of the common practice steps performed towards food-borne pathogens' cluster detection.

Methods: We have reviewed the literature for factors impacting on the genetic dissimilarity of the isolates within an outbreak, using an information foraging approach. We used the approach of cognitive mapping to describe the relations between the various factors. The identified factors have been depicted in an influence diagram, using the R package DiagrammeR.

Results: We have identified a series of factors impacting on the observed genetic dissimilarities. Of these, the average substitution rate and environmental stability, which in turn determines the outbreak duration, could already be used in cluster refinement. Other factors, such as strain or niche specific mutation and recombination rates, or bacterial fitness, require still a significant amount of research for parametrization of future quantitative models incorporating them.

Conclusions: We underline the complex relations between the biological and ecological factors at play in the evolution of some of the most common food-borne pathogens, the standards chosen. The conceptual model is a first step in developing and evaluating new algorithms of cluster definition/detection. as it pinpoints key aspects of the biology of the bacterial pathogens that underlie the observed clustering. Implementation of quantitative models using the identified factors for cluster refinement may be particularly useful for pathogens for which the exposure is high (e.g. *Salmonella enterica*).

[P033] 16S RRNA MICROBIAL COMMUNITY ANALYSIS AND RELATIONSHIP WITH SALMONELLA SUPER SHEDDER STATUS IN PIGS

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16S rRNA microbial community analysis and relationship with Salmonella Super shedder status in pigs

Intestinal microbiota species richness and relative abundance can be linked with the health state of the animals. Recent studies have uncovered the importance of host heterogeneity in infection with zoonotic pathogens, and it has been shown that a minority of the infected individuals are responsible for the majority of the infections (known as 'super-shedders'). A better understanding of the composition of the microbiota of super-shedders may facilitate targeted interventions with, for example, pre and pro-biotics, to reduce colonization and shedding.

Aim: to investigate whether there was any association between *Salmonella* shedding status in pigs and microbiota heterogeneity.

Methods: 16s rRNA community profiling analysis was conducted on 458 samples (faeces from live animals and GI contents collected at *post-mortem* examination). The association between microbiota species richness and relative abundance, with husbandry, clinical parameters, and *Salmonella* shedding status, were investigated using an established bioinformatic pipeline for the statistical analysis of microbial community (Qiime2).

Results: The study detected small, but statistically significant differences in the bacterial species richness between sample types, between the different groups of pigs and also according to the shedding status (versus the control group) with implications for our understanding and potential mitigation of foodborne zoonoses. Further analysis using machine learning and mathematical modelling tools are under development to further assess the relationships between bacterial communities.

Conclusions: The statistical significant difference found increased our understanding and potential mitigation of foodborne zoonoses. Machine learning approach and matematical modelling (under development) will further assess the relationship between bacterial communities.

[P034] PRESENCE OF KLEBSIELLA PNEUMONIAE IN FOOD, FAECES AND ENVIRONMENTAL SAMPLES TESTED FROM 2018 TO 2020

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Aim: A broad sampling from 2018 to 2020 was done to define the ecology of *Klebsiella pneumoniae* (Kp), one of the most problematic multidrug resistant organisms.

Methods: For the detection of Kp strains an harmonized method was used: enrichment in buffered peptone water (BPW) at 37°C/24h and plating on SCAI agar at 44°C/48h for foods and environmental samples. A further enrichment in Luria broth+ampicillin at 37°C/24h was performed for human and animal faeces. A total of 963 samples were analyzed in the broad sampling, in 2018 and 2019: 439 foods (chicken meat, RTE salads and vegetables, live bivalve molluscs, milk and dairy products), 58 environmental samples, 208 animal faeces and 28 human faeces. A deep sampling was carried out in 2020 consisting in seawater (60), onions (110) and live bivalve molluscs (60).

Results: Among tested foods, chicken meat had the highest prevalence of Kp (32/160-20%), while RTE salads and vegetables had 7 out of 94 positive samples (7.4%). In animals 20 out of 208 were positive (9.6 %). Samples from environmental sources, waste water and soil, showed 37 out of 58 positive samples (63.8 %). Deep sampling of seawater and molluscs highlighted 22 out of 60 positive samples (36.7%) and 13 out of 60 (21.7%) respectively. Onions samples showed 14 out of 110 positive samples for Kp (12.7%).

Conclusions: Our results highlighted the presence of Kp in animals, environment and vegetables like onions. Furthermore, the presence in food must increase its surveillance to avoid the trasmission to consumers.

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[P035] ASSESSMENT OF THE EFFECTIVENESS OF PRE-HARVEST MEAT SAFETY INTERVENTIONS TO CONTROL FOODBORNE PATHOGENS IN BROILERS AND PIGS: SYSTEMATIC REVIEWS

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Aim: Stakeholder cooperation along the food chain is the central element of the risk-based approach in meat hygiene. The aim of this study was to assess the effectiveness of pre-harvest meat safety interventions by means of systematic literature reviews for broilers and pigs.

Methods: The inclusion and exclusion criteria for the systematic literature reviews have been the same for both animal species except for the search timeframe: broilers: 2015–2020 and pigs: no time limit. EFSA Scientific Opinion's on the public health hazards related to meat from broilers and swine were the basis for the pathogens' selection (EFSA 2011, 2012).

Results: Fifty-one studies regarding *Campylobacter* spp., *Salmonella*, VTEC, ESBL-AmpC *Escherichia coli*, and *Clostridium perfringens* were included in the broiler review. Research mostly focused on *Salmonella* and *Campylobacter* spp., with biosecurity and management interventions having mixed outcomes. The effectiveness of feed additives remains controversial. Overall, studies on recent developments of novel pathogen-specific immunisation strategies are lacking.

In pigs, 51 studies were retained contemplating five pathogens. *Salmonella* was the most investigated (n=42 studies) with feed and/or water treatments and vaccination being the most researched interventions and having positive results. Overall, high health status coupled with good management and biosecurity are effective to control most foodborne pathogens in pork.

Conclusions: Both in broilers and pigs, research on many pathogens was scarce or with focus on epidemiology/source-attribution studies. This may be partly explained because several of these pathogens are frequently controlled by post-harvest interventions.

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[P036] EXAMINATION OF FISH PRODUCTS FROM POLISH MARKETS FOR DETECTION OF ANISAKIS SIMPLEX ANTIGEN.

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Aim: The aim of our study was examination of fish products from Polish markets for detection of *Anisakis simplex* antigen by CL sandwich ELISA assay.

Methods: A total of 259 products were tested to assess the occurrence of *A. simplex* antigen in seafood products from Polish markets.We tested products from different fish/ squid species and type of product processing. As a diagnostic test we used CL sandwich ELISA described previously by Kochanowski et al.(2020).

Results: Among tested samples, 28% of which were positive. More than half of the positive samples (n = 39) comprised the following smoked fish products: mackerel, herring, cod, and hake. Other positive samples were found in marinated herrings, canned cod livers, canned mackerels, and surimi sticks. No positive food samples were detected in the following species of tested products: tuna, Atlantic argentine, sardine, sprat, squid, and anchovy.

Conclusions: Based on our findings, it can be concluded that around 28% of processed seafood products in Polish markets contain *A. simplex* antigen and, therefore, may pose potential allergic hazards for sensitized consumers. Further studies are necessary to estimate the occurrence of *A. simplex* proteins in a larger group of different seafood products.

References: Maciej Kochanowski, Mirosław Różycki, Joanna Dąbrowska, Jacek Karamon, Jacek Sroka, Ewelina Antolak, Aneta Bełcik, and Tomasz Cencek. Development and Application of Novel Chemiluminescence Immunoassays for Highly Sensitive Detection of Anisakis simplex Proteins in Thermally Processed Seafood. Pathogens, 2020, 9: 777.

[P037] DEVELOPMENT AND VALIDATION OF CHEMILUMINESCENCE ELISA METHODS FOR DETECTION OF ANISAKIS SIMPLEX PROTEIN IN FOOD

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Aim: The aim of our study was the development and validation of high-sensitivity chemiluminescent (CL) immunoassays for the detection of A. simplex proteins in processed seafood

Methods: Validation of developed assays (CL sandwich ELISA and CL competitive ELISA) was performed with food extracts from trout fillets. Spiked and non-spiked samples with native A.simplex were prepared (n = 63). All samples were heated in a thermomixer at 100 °C for 60 min. Additionally, 72 spiked autoclaved canned fish products (fish in sunflower oil, fish in tomato sauce, and fish without additives) were used for evaluation of the usefulness of the methods.

Results: The limit of detection (LOD) of CL-S-ELISA (0.5 ng/mL) was 10 times better than that of CL-C-ELISA (5 ng/mL). CL-S-ELISA had lower intra- and inter-assay variations (better precision), compared to CL-C-ELISA. The calculated area under the ROC curve (AUC) values were high for both assays, indicating that both CL-ELISA methods were highly accurate. A. simplex L3 larvae were detected in all autoclaved spiked products using both CL-ELISA methods. All non-spiked samples were negative in both assays. No differences in test performance were found for the examination of the following matrices: fish without additives, fish in tomato sauce, and fish in sunflower oil.

Conclusions: Both developed CL-ELISA methods were sufficiently sensitive for the examination of heat-processed fish products; however, CL-S-ELISA had better performance than CL-C-ELISA. The results of our investigations showed that CL-immunoassays are highly effective tools for A. simplex antigen detection, which is useful for food control laboratories.

[P038] TROPISM AND PERSISTENCE OF TOXOPLASMA GONDII: FROM PORK CARCASS TO DRY SAUSAGE

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Aim: *Toxoplasma gondii* is an important zoonotic foodborne parasite. Amid the possible transmission routes, meat of infected animals appears to be a major source of infection in Europe. In France, pork is the most consumed meat, especially in the form of dry sausage. However, the risk of parasite transmission via the consumption of specific processed pork products is unknown, mainly since processing will affect viability but may not entirely inactivate all *T. gondii* parasites.

Methods: We investigated the predilection sites and distribution of the parasite in selected tissues of 7 pigs experimentally infected with 1000 oocysts/tissue cysts and 2 naturally infected pigs by means of MC-PCR. Muscle tissue of experimentally infected pigs was used to evaluate the impact of the manufacturing process, including different concentrations of nitrites (0–120 ppm) and NaCl (0–26 g/kg), ripening (2 days/16–24°C) and drying (up to 30 days/13°C) of dry sausage with a combination of bioassay, qPCR and MC-PCR.

Results: DNA of *T. gondii* was detected in 48.1 % (13/27) of muscle samples by MC-PCR. In dry sausages, 94.4 % (51/54) of samples were positive for *T. gondii* by PCR, however, none were positive by mouse bioassay.

Conclusions: Results suggest an uneven distribution or a tissue cyst concentration below the detection limit in the pig carcasses examined. Even in dry sausage without added NaCl and nitrites, no viable *T. gondii* were detected. Results will be used in quantitative microbiological risk assessment for *T. gondii*.

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[P039] TOXOPLASMA GONDII PREVALENCE IN ANIMALS IN EUROPE: SYSTEMATIC REVIEW

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Aim: *Toxoplasma gondii* is a zoonotic parasite of importance to both human and animal health. The parasite has various transmission routes, and meat of infected animals appears to be a major source of infection in Europe. Therefore, we aimed to assess the *T. gondii* prevalence in Europe in a selection of its animal host species.

Methods: For the first time in Europe, a systematic literature review was performed for 37 animal species, ranging from domestic cats and livestock to wildlife. The systematic review was conducted according to PRISMA guidelines. Study selection was performed independently by 20 scientists from 13 countries across Europe, using the online tool Cadima. Peer-reviewed articles published in English since 2000 were eligible, based on specified criteria including natural infection, detection of *T. gondii*, and host species.

Results: A total of 1985 publications were retrieved. After screening titles, abstracts and full texts, 275 articles met the inclusion criteria for data extraction. The included studies provide data on pigs (55), felids (48), sheep (39), wild boars (30), wild ruminants (29), goats (22), cattle (20), lagomorphs (13), equids (12), poultry (11), wild birds (10) and buffaloes (1).

Conclusions: The data provide unique overview and strong input for assessment of source attribution, and can thereby aid the development of effective prevention strategies. The data, with emphasis on regional and age-related *T. gondii* prevalence estimates, will be analyzed by Bayesian hierarchical modeling and used as input data in a multi-country quantitative microbiological risk assessment within TOXOSOURCES project.

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[P040] INFLUENCE OF ORAL ADMINISTRATION OF LACTOBACILLUS REUTERI PROBIOTIC STRAINS AND GOS PREBIOTIC ON THE PRESENCE OF SALMONELLA SPP. IN BROILERS

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Aim: The aim of this study was to investigate the effect of the addition of probiotics Lactobacillus reuteri SAP 2114 and L. reuteri SAP 2115, alone or in combination with galacto-oligosaccharides (GOS - Clasado) prebiotic on the growth rates of broilers and the presence of pathogenic intestinal bacteria.

Methods: The studies were performed using 4 experimental groups, each consisting of 18 160 broilers (day old to slaughter age). Broth cultures of probiotics Lactobacillus reuteri SAP 2114 L. reuteri SAP 2115 and GOS as a prebiotic were tested in the studies. Each broiler received at least 1x109 CFU / day of the probiotics via the water system. The number of lactic acid organisms in the faeces were enumerated using De Man, Rogosa and Sharpe agar, the presence of Salmonella according to the ISO 6579-1: 2017 methodology.

Results: Indicators of production efficacy, such as food conversion ratio, the incidence of intestinal disorders and morbidity and mortality were monitored. The numbers of Lactobacillus spp. in broilers in group 3 (both probiotics plus prebiotic) was the most stable and was found to be above 7.8 CFU / g and statistically significant comparing to control group ($P \leq 0.01$). Post slaughter sampling detected Salmonella Infantis in group 2 treated with the L. reuteri SAP 2115 probiotic and group treated with both probiotics and the prebiotic. Group 1 which received just the L. reuteri SAP 2114 probiotic was free of Salmonella Spp.

Conclusions: Treatment of broilers with Lactobacillus reuteri SAP 2114 resulted in the absence of Salmonella in faeces at slaughter. A combination of both probiotics and a prebiotic resulted in a reduced incidence of intestinal disorders and better food conversion ratios compared to the control group.

[P041] COMPARING ZOONOTIC PATHOGENS BETWEEN BROWN (RATTUS NORVEGICUS) AND BLACK (RATTUS RATTUS) RATS IN THE NETHERLANDS USING 16S NEXT-GENERATION SEQUENCING OF KIDNEY SAMPLES

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Aim: The aim of this study was to investigate whether variation in rat populations (in urban versus rural areas, and between brown and black rats) is reflected in their pathobiome and in host factors associated with certain zoonotic pathogens. Besides that, we wanted to assess the usability of next-generation sequencing (NGS) for surveying and identifying zoonotic bacteria in rats.

Methods: To test this, black and brown rats collected for other studies from 2013-2018 were selected, based on species, area type in which they were trapped, etc. From each rat, DNA from a kidney sample was used for NGS of the bacterial 16S rRNA V3/V4 region. Data was analyzed in R using the packages DADA2 and phylosec.

Results: The use of a sample with low pathogen load, such as a kidney sample (in contrast to e.g. a gut sample), is a fairly new and different approach in NGS. The work is ongoing and the latest results will be presented, discussing the difficulties that arise with working with low pathogen load samples as well as the differences in pathogen diversity between both rat species and the different area types (including α-diversity and β-diversity plots). These results can be used to identify host-pathogen-ecology combinations that form a relatively higher risk for human health.

Conclusions: No conclusions available yet (analysis is still ongoing).

[P042] IDENTIFICATION OF DATA CURRENTLY USED IN QUANTITATIVE MICROBIAL RISK ASSESSMENT

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Aim: Cross-sectoral framework for quality Assurance Resources for countries in the European Union (CARE) project is an Integrative Project of the One Health European Joint Programme (OHEJP). The objective is to set standards in the future, which will strengthen already existing systems for proficiency testing, reference material and quality/availability of data necessary for risk assessment. One of the objectives of Work Package 4 is to identify which (meta-) data is currently used in quantitative microbial risk assessment (QMRA) and meant to be shared.

Methods: A survey intended for OHEJP members and broader was launched from September 2020 to March 2021 to collect informations about existing QMRA studies from the last 5 years. The questionnaire was set up on Sphinx online software and was organized into 31 main questions divided into 3 sections. The first section corresponded to the identification of the respondent, followed by the description of the QMRA study and the data included.

Results: The survey revealed that the main hazards assessed were pathogenic *E. coli*, *Listeria monocytogenes* and *Salmonella* spp.. While the starting point of the QMRA studies is diverse, the endpoint is mostly at the consumer stage. Three types of data sources have been described: data produced by the risk assessor organisation, data from literature and data from a scientific network. Part of this data is publicly available but not recorded on a dedicated platform.

Conclusions: This study contributes to an inventory of high quality data available. Harmonized and controled vocabulary would improve their accessibility.

Funding: This work is funded by One Health EJP founding. It is part of the Work Package 4 of the IJP CARE.

[P044] MOLECULAR DETECTION AND DIFFERENTIATION WITHIN BRUCELLACEAE FAMILY IN URBAN AND RURAL WILDLIFE

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Aim: Within the Brucellaceae family highly pathogenic and zoonotic *Brucella* species are classified, alongside opportunistic or even environmentally *Ochrobactrum* species. The aim of IDEMBRU project is creation of a toolkit for identification of new *Brucella* species and unsuspected or emerging reservoirs. As *Brucella* and *Ochrobactrum* share ~80% genetic similarities, a new multiplex PCR was created to distinguish between two genuses.

Methods: To detect *Brucella spp.* a specific PCR for IS711 was used, to which a new *Ochrobactrum spp* target was added. New multiplex PCR was tested on tissues collected from various wild animal species held in the Paris Zoo, representing the urban areas, still with potential sources for *Brucellaceae* reservoirs. While samples originating from foxes and wild boars across French forests, obtained through bovine tuberculosis surveillance system in wildlife, represent reservoirs in forest biotopes with different feeding habits.

Results: The new multiplex PCR was able to detect referent *Ochrobactrum* strains and differentiate from panel of selected classical or atypical *Brucella* species. Using this multiplex PCR, no positive animals (of 65) were detected in the Paris Zoo. Out of 388 foxes, five (1.3 %) were *Ochrobactrum spp.* Positive, while *Brucella spp.* DNA was not detected. On the contrary, from 60 wild boars, 21.7 % were *Brucella spp.* and 0.17 % were *Ochrobactrum* positive.

Conclusions: The new multiplex PCR assay is highly specific in distinguishing between *Brucella spp.* and *Ochrobactrum spp*. The absence of both genuses from urban environement helps focus the IDEMBRU project on rural areas and forest biotopes.

[P045] TOOL DEVELOPMENT FOR THE DETECTION AND IDENTIFICATION OF CRYPTOCOTYLE METACERCARIAE FROM FISH: DIGESTION METHOD, VIABILITY STUDY, MORPHOLOGICAL AND MOLECULAR IDENTIFICATIONS

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Aim: Fish harbor a wide variety of parasites. To date, many are still widely unknown, especially for their zoonotic potential. Some Opisthorchioidea parasites such as *Clonorchis* and *Opisthorchis* are recognized as zoonotic and responsible of numerous human pathologies. *Cryptocotyle* belongs to this superfamily and induces black spot disease on fish. Human can potentially be infected by consumption of parasitized fish. However, so far, its impact on human health is still unknown and few publications exist dealing with its recovery, identification and distribution among commercially important fish.

Methods: The present study describes tool development for the quantification of *Cryptocotyle* infection, including optimization of digestion, assessment of viability of metacercariae after fish death, and morphological and molecular identification.

Results: Orbital stirring of the pepsin digestive solution was found less destructive for metacercariae and allowed better quantification of infection. Viable metacercariae were recovered up to 15 days after host death. However, metacercariae might be morphologically identified up to 8 days post fish death, allowing scheduling of experiments. *Cryptocotyle* from whiting and Atlantic cod caught off the Channel and from Danish marine waters, were characterized from a morphological point of view with microscopic observations and from a molecular perspective with Sanger sequencing of fragments of *cox1* gene and *ITS* region.

Conclusions: Optimized protocols for a reliable quantification, detection and identification of *Cryptocotyle* were performed. This allowed the first description and identification of *C. lingua* metacercariae from whiting from the Channel. The methods will allow big scale epidemiological studies on *Cryptocotyle* prevalence, intensity and identification to be realized in the future.

[P046] SYSTEMATIC REVIEW AND META-ANALYSIS OF RISK FACTORS FOR TOXOPLASMA GONDII INFECTION IN LIVESTOCK RAISED FOR HUMAN CONSUMPTION

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Aim: In this study, we aimed to determine the relationship between potential on-farm risk factors and *Toxoplasma gondii* infection in pigs, bovines, sheep, goats, chickens, turkeys and horses, through systematic literature review and meta-analysis. *Toxoplasma gondii* is a zoonotic protozoan that can infect humans following the ingestion of tissue cysts in raw or undercooked meat from infected animals, or oocysts shed by infected cats. Understanding the risk factors for infection in livestock species, which can act as a source of human infection, will help inform control and prevention strategies aimed at reducing the burden of disease in humans.

Methods: Following an a priori developed protocol, we systematically searched the Embase, Medline and Biosis Previews databases to identify potentially relevant literature in two stages, covering 1994 to 2018 and 2018 to November 2020. Identified records were screened against pre-determined selection criteria through three increasingly rigorous screening rounds. Data from the remaining records were then extracted, cleaned and analysed.

Results: Of the 868 unique records screened for eligibility and relevance during the first stage, 134 were selected for data extraction and inclusion in the review. Of these, 131 were cross-sectional studies, two were case-control studies and one was a census. Most of the studies were conducted in Europe (n=52) and South America (n=36) and all listed host species were represented. First results of descriptive and meta-analysis will be presented.

Conclusions: The results will improve understanding of risk factors for *Toxoplasma gondii* infection and help inform public health control programs and risk analysis.

Funding: This work was done as part of ORION project in collaboration with TOXOSOURCES project, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

Earlier work from another project contributed data in the form of potentially relevant literature from 1994 to 2013. That work was supported by the European Food Safety Authority and conducted by a consortium within the framework of project number GA/EFSA/BIOHAZ/2013/01 entitled "Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat" (€ 400.000). Some of the results in this work are based on the results obtained in the framework of this mentioned project and are published under the sole responsibility of the authors. The output shall not be considered as an EFSA output. The UK component of the work was supported by the Food Standard Agency United Kingdom [project FS517004].

[P047] IMPACT OF ZINC ON HORIZONTAL GENE TRANSFER OF ANTIMICROBIAL RESISTANCE DETERMINANTS IN ENTEROBACTERIACEAE

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Aim: Antimicrobial resistance (AMR) is a global public health concern. There has been an increased focus on the role primary production can play in the development and dissemination of AMR and factors which can influence it. Heavy metals are utilised within animal production for nutritional and medicinal purposes and may influence AMR through co-selection or cross-resistance. It is known that AMR can be acquired via mobile genetic elements, but the impact of heavy metals on AMR transfer is limited. This study investigated the impact of zinc on horizontal gene transfer (HGT) in Enterobacteriaceae.

Methods: A conjugation experiment was designed, using an extended spectrum beta-lactamase-producing *Escherichia coli* strain as a donor and three *Salmonella* strains as recipient strains. To aid selection, rifampicin-resistant recipient strains were raised. AMR profiles of all strains were determined using disk diffusion assays and minimal inhibitory concentrations for zinc nitrate were measured. Donor and recipient strains were incubated together in the presence and absence of zinc to investigate its effect on conjugation frequencies. Transconjugants were further analysed using culture-based methods and whole genome sequencing.

Results: Transconjugants were successfully obtained for all three combinations of donor and recipient strains. One of three recipients showed significantly higher transconjugant numbers ($p < 0.05$) in the presence of zinc. Conjugation frequencies varied significantly between recipient strains.

Conclusions: The results showed that zinc can have an impact on HGT in Enterobacteriaceae. Plasmid type and genetic factors may play an additional role.

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[P048] ELICITING ACCEPTABLE INVESTMENTS IN SENSITIVITY OF ONE HEALTH SURVEILLANCE

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Aim: Our aim is to estimate the willingness to pay (WTP) and willingness to accept (WTA) values for a one health surveilance with a range of expected sensitivity levels and uncertainty. Our hypothesis is that we will find a difference in WTP and WTA, where people expect to receive higher payments to forgo sensitivity, compared to paying for that sensitivity. We also expect to find that respondents will have higher WTP (WTA) when baseline sensitivity is high (low).

Methods: Each respondent will be randomly allocated to a questionnaire which will include questions either in relation to WTP or WTA. Participants will be presented with two existing sensitivity values which will prompt them to indicate how much they would be willing to invest/divest for an increase/decrease in sensitivity. Subsequent questions will ascertain WTP and WTA values when faced with different levels of uncertainty to assess risk aversion.

Results: We will be able to present the distribution of the preferential investments in the initial increase and compute the difference between WTP and WTA scenarios. We will also be able to show how uncertainty altered the WTP and WTA for respondents in each scenario.

Conclusions: Our study acts as a benchmark to inform cost-effectiveness analysis of decision making by policymakers. This study can additionally act as a basis for further investigations into what value we give to disease surveillance and how much stakeholders are willing to invest in the sensitivity of information.

[P049] FAECAL CARRIAGE OF EXTENDED SPECTRUM B-LACTAMASE PRODUCING AND CARBAPENEM RESISTANT ENTEROBACTERALES IN WATER USERS IN IRELAND: A POINT PREVALENCE STUDY

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Aim: Exposure to antimicrobial resistant (AMR) bacteria in natural recreational water may represent a substantial public health risk through gut colonisation and onward transmission to vulnerable individuals. We aimed to compare the prevalence of gut colonisation in regular water users (WU) and non-water users (NWU) in Ireland, with carbapenem-resistant Enterobacterales (CRE) and extended spectrum beta-lactamase producing Enterobacterales (ESBL-PE).

Methods: Between September and December 2020, 141 faecal samples were collected from 70 WU and 71 NWU and cultured on CHROMagar mSuperCARBA™ and Brilliance ESBL to screen for CRE and ESBL-PE respectively. Colonies of interest were identified by MALDI-TOF and antimicrobial susceptibility testing (AST) was performed according to EUCAST criteria. Real-time PCR was used to detect bla_{CTXM} group 1, bla_{CTXM} group 2, bla_{CTXM} group 9, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, bla_{NDM}, and bla_{KPC} genes.

Results: Of 56 suspect CRE/ESBL-PE that grew on selective agars, AST phenotypically identified 5 CRE, 10 presumptive ESBL-PE and 1 isolate with combined CRE/ESBL-PE phenotype. PCR confirmed blaCTXM in 8 isolates; 6 bla_{CTX-M} group-1 *E. coli* (all in NWU) and 2 bla_{CTX-M} group-9 *E. coli* (all in WU). No carbapenemase gene was detected amongst the 6 CRE isolates.

Conclusions: This ongoing study found that 8% and 4% of participants are colonised with ESBL-PE and CRE, respectively. There was no significant difference in ESBL-PE/CRE colonisation rate between WU (7%) and NWU (15%), (χ^2 P=0.12). Colonisation of healthy people with CRE is particularly concerning. Genomic analysis may reveal the resistance mechanisms involved. Further sampling and participant survey analysis may identify significant risk factors for colonisation.

Keywords: Bathing water, antibiotic resistant bacteria, CRE, ESBL, colonisation, public health.

Funding: This research was carried out as part of the PIER (Public health Impact of Exposure to antibiotic Resistance in recreational waters) project. This project is funded under the EPA Research Programme 2014-2020. The EPA Research Programme is a Government of Ireland initiative funded by the Department of Environment, Climate and Communications. It is administered by the Environmental Protection Agency, which has the statutory function of co-ordinating and promoting environmental research

[P050] FAST AND EASY FIELD-DEPLOYABLE SARS-COV-2 VIRUS INACTIVATION FOR DOWN-STREAM ANALYSIS

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Aim: Point-of-incidence (POI) diagnosis enables a quick response to emerging infectious diseases. Reducing transmission risk during sample handling is paramount. Previous studies have shown virus inactivation abilities of the highly cytotoxic MagNA Pure Lysis Binding (MPLB) buffer used for nucleic acid extraction and purification. In this study, we show rapid inactivation of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using a modified published protocol [1].

Methods: For virus inactivation, two human SARS-CoV-2 isolates of 10^5 and 10^6 Tissue Culture Infectious $Dose_{50}$/ml were incubated 1:1 with MPLB-buffer or PBS (positive controls) for 20 minutes. The mixture was diluted to non-cytotoxic MPLB-buffer concentrations (determined empirically) and incubated on VeroE6 cells. Supernatant and cells were harvested at multiple time-points in biological duplicates with technical triplicates. We used reverse transcription quantitative-PCR (RT-qPCR) with a standard curve for quantification. To ensure inactivation, we serially passaged supernatant from a 144h culture and measured active replicating virus at the 24h time-point with a SARS-CoV-2 E-gene RT-qPCR [2].

Results: MPLB-buffer incubated SARS-CoV-2 samples were non-infectious in the 10^{-4} MPLB-buffer dilution, except three samples indicated by median ct-value 39.8 (IQR=4.8) from the 10^5 $TCID_{50}$/ml stock. The control grew efficiently with median ct-values 11.8 (IQR=1.8) for cells and 16.1 (IQR=1.8) for supernatant. The sub-genomic RT-qPCR for the 24h time-point showed no evidence for virus replication, including the three exceptive samples.

Conclusions: We show that MPLB-buffer can reduce SARS-CoV-2 titers at least 2-log units. With this protocol, we intent to facilitate POI-diagnosis and promote field research without risk of infection.

Refereneces:
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[P051] ONE HEALTH EJP - RADAR MODEL INVENTORY: A USER-FRIENDLY TOOL FOR ANNOTATING AND EXCHANGING MODELS

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Aim: The lack of a harmonized model exchange formats among modelling tools impedes communication between researchers since the exchange and usage of existing models in various software environments can be difficult. The RaDAR model inventory aims to provide a platform to exchange models among professionals utilizing the Food Safety Knowledge Markup Language (FSK-ML) [1] as a harmonized model exchange format.

Methods: Agile software development.

Results: FSK-ML defines a framework that encodes all relevant data, metadata, and model scripts in an exchangeable file format. However, the creation of such a file can be a time-consuming and difficult process. To increase the usage of the FSK standard, we developed the RaDAR model inventory web application that targets the process of creating an FSK-ML file for the end user. Our inventory aims to be a user-friendly tool that allows users to create, read, edit, write, execute and compile FSK-ML files within the web browser. The possibility of sharing models with the public or a specific group of people facilitates collaboration and the exchange of information. Since the RaDAR model inventory is based on the open-source technology of Project Jupyter [2], it can support nearly all relevant programming languages executed within a reproducible cloud-computing environment.

Conclusions: The intuitive nature of the RaDAR model along with its wide range of features reduce the threshold for contribution to a harmonized model exchange format and eases collaboration. The RaDAR model inventory can be accessed at https://ejp-radar.eu.

References:

Miguel de Alba Aparicio et al. (2018) FSK-Lab – An open source food safety model integration tool

Perez et al. (2015) Project Jupyter: Computational narratives as the engine of collaborative data science

[P052] AUTOMATED DETECTION OF AMR TARGET GENES USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

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Aim: Loop-mediated isothermal amplification (LAMP) technology presents a relatively low-cost technique for the rapid detection of pathogens and antimicrobial resistance (AMR), by amplifying associated genes to levels that can be readily detected without the need for complicated equipment. Therefore, LAMP is a suitable technique for a portable detection tool in the field to assess threats to human and animal health posed by AMR. Currently, fluorescence and colorimetric LAMP detection are the most commonly used platforms. However, user subjectivity, training and variable conditions can interfere with the diagnostic accuracy. To address this, we propose a machine learning approach for automated detection.

Methods: LAMP assays targeting AMR genes: KPC, OXA-48 and mcr-1, were designed and evaluated in real-time using fluorescence imaging within 30 min. Samples were manually labelled as either positive or negative with reference to test controls, and predictor variables consisted of the amplification signal and annealing temperatures. These samples were used to train a deep convolutional neural network (CNN) as a predictive model.

Results: In total, 56 data samples were used for model training and evaluation. We applied leave-one-out cross-validation to estimate the performance of the CNN. After training, we obtained a test accuracy of 98.2 ± 0.6 %; suitably, the model was robust against challenging samples indicating false positives.

Conclusions: Automating the detection of LAMP reactions provides more objectivity in data interpretation, speeds up the data analysis process and enables integration with mobile detection. We expect this approach to be useful as part of an on-site diagnostic toolkit for the detection of AMR.

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[P053] ECOLOGICAL MODELLING OF MICROBIAL COMMUNITIES SUBJECT TO PERTURBATION

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Aim: It is increasingly clear that gut microbiota plays a pivotal role in health, for example by providing protection against pathogenic invasion; however, the interactions between commensal bacteria is complex and not well understood. Therefore, we devise a mechanistic model to describe the temporal dynamics of microbial communities. Our main goal is to predict community stability in response to time-varying external perturbations, e.g. antibiotic treatment, and to identify the conditions under which catastrophic shifts in their composition might occur.

Methods: Time-series 16S rRNA sequencing data is used as the input for our model, including bacterial abundances and total biomass for absolute counts. We apply the generalised Lotka-Volterra model (gLV), as is frequently used to describe ecological systems, in order to predict community compositional changes over time. We also model external perturbation that mimics antibiotic administration, and infer the model parameters using Bayesian linear regression.

Results: We used experimental data from Stein et al. 2013 [1] to infer bacterial growth, interaction and antibiotic susceptibility parameters for the gLV model, restricted to the top 10 most abundant genera to avoid overfitting. From exploratory simulations, we identified a tipping point of the community in response to external perturbation: resulting in a persistent change in its composition from which it was unable to recover.

Conclusions: Ecological modelling of microbial communities affords additional insight into their stability, which cannot easily be determined from experimental studies alone. Our next step is to explore the impact of different perturbation regimes, with the long-term goal to model data obtained from chemostat experiments.

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[P054] COLLECTION OF PROTOCOLS FOR THE DETECTION AND CHARACTERISATION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC), ENTEROTOXIGENIC E. COLI (ETEC), CRYPTOSPORIDIUM SPP. AND ANTIMICROBIAL RESISTANCE IN SALMONELLA AND CAMPYLOBACTER

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Aim: The Work-Package (WP) 4 of One Health EJP project OH-Harmony-CAP (One Health Harmonisation of Protocols for the Detection of Foodborne Pathogens and AMR Determinants) is devoted to the collection, assessment and ranking of protocols for the detection and characterization of model organisms, with the aim to propose harmonised procedures. The selected organisms are Shiga toxin-producing *Escherichia coli* (STEC), Enterotoxigenic *E. coli* (ETEC), *Cryptosporidium* and antimicrobial resistance in *Salmonella* and *Campylobacter*.

Methods: Laboratory protocols were collected by sending a request to laboratories of different networks, including OH-Harmony-CAP partners, national reference laboratories and public health laboratories. Groups of experts for each model organism were formed. Evaluation tables were created to gather and summarize the protocols.

Results: Overall, 23 institutions operating in public health or veterinary and food safety field, responded to our request and submitted their laboratory protocols. The files shared included different types of documents, such as standard operating procedures, published works, standard methods, and general information on the protocols applied. The groups of experts filled in the evaluation tables devoted to each pathogen.

Conclusions: A preliminary evaluation of the collected protocols confirms that there is room for harmonisation. The next step will be to compare the collected protocols and to propose a harmonized procedure for each model organism. The final objective will be to share the harmonized protocols in a technical report and to use them for the practical trainings scheduled for WP5 of the OH-Harmony-CAP project.

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[P055] DECREASED MORTALITY IN PATIENTS WITH BLOODSTREAM INFECTIONS CAUSED BY MULTIDRUG RESISTANT ESCHERICHIA COLI

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Aim: Bloodstream infections, especially those resistant to antibiotics, are on the rise. *Escherichia coli* is the most common microorganism implicated in these serious infections and multidrug resistant isolates are frequently isolated. However, there remains a paucity of data relating to the risk factors associated with these infections. Therefore, a 12-month longitudinal study was conducted to decipher potential risk factors.

Methods: The study integrated the genomic, phenotypic and clinical characteristics of a panel of 111 *E. coli* isolates recovered from the blood of patients at a South London UK hospital between 2017 and 2018.

Results: Most bloodstream infections were community-acquired and derived from a urinary tract source, mainly affecting patients over 65 years of age. Antibiotic resistance incidence varied significantly throughout the year and was associated with previous antibiotic treatment and specific sources of infection, especially intra-abdominal. ST131 was the most common multi-locus sequence type, frequently harbouring antibiotic resistance genes and plasmids, and the presence of IncFI plasmids was associated with isolates being multidrug resistant.

All-cause 30-day mortality was significantly associated with the source of infection and, although not significantly, mortality was higher than the 13% average in patients with isolates belonging to ST131 and ST69, in age groups of 49-64 and 79-98, with hospital-acquired infections, male patients and, unexpectedly, with non-multidrug resistant isolates.

Conclusions: This study highlights the need of integrating molecular and clinical data at a larger scale to better understand the main factors driving mortality in patients with bloodstream infections and inform the design of effective interventions.

Funding: This study is funded by One Health EJP "ARDIG"

[P056] AN INNOVATIVE MICROFLUIDIC QPCR PLATFORM FOR HIGH THROUGHPUT TESTING OF BRUCELLA SP. AND OCHROBACTRUM SP. IN ENVIRONMENTAL SAMPLES

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Aim: Brucellosis is a worldwide zoonosis, that infects mammals primarily, but new hosts and species are regularly identified. The IDEMBRU project has the aim to develop tools for the identification of new species from environment and atypical hosts. One of these tools will be a high throughput qPCR microchip based on the Fluidigm technology. This chip will be a powerful tool for a global screening of unknown samples.

Methods: With this chip, 192 samples can be tested against 24 targets, allowing the results of 4608 data points (duplicates) within one single reaction. Specific targets related to *Brucella* and *Ochrobactrum* genus will be used. A preamplification step will be performed (primers/total DNA/PreAmp Master Mix). After a conventional PCR step, the pre-amplified DNA will be used to look for specific targets on the microchip and will give a global screening of the sample with the qualitative results (presence/absence) of specific species.

Results: Specific design for *Brucella* and *Ochrobactrum* have been tested in qPCR and gave promising results. The next steps will be to transfer the technology of qPCR to the microfluidic level.

Conclusions: The microfluidic qPCR developed within this project will be a powerful screening tool to detect the presence of bacterium of interest in a sample.

[P057] ADVANCES IN UNDERSTANDING THE ENVIRONMENTAL DRIVERS OF HUMAN SALMONELLOSIS USING MODELLING

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Aim: The higher incidence of *Salmonella* infections consistently reported in humans in the summer season suggests that the environment has an important role as a modulator of infections. We aim to explain these observed incidence patterns by identifying the main environmental drivers and their interactions.

Methods: Based on evidence linking the growth of *Salmonella* in eggs and chicken meat to temperature, and assuming a correlation between the detected cases of salmonellosis and the bacterial load in the food, we developed a mathematical model to simulate the incidence of the disease using daily mean air temperatures recorded in England and Wales during the years 1989 to 1995.

Results: Our incidence estimates for human salmonellosis closely reflect the number of reported human cases of *Salmonella* from Public Health England. This suggests that modelling bacterial growth in eggs and fresh chicken products alone is sufficient to adequately predict the association between cases of salmonellosis and seasonal environmental factors.

Conclusions: Chicken and eggs and the effect that temperature exerts on the growth of Salmonella in them have a remarkable association with the incidence of salmonellosis. We expect that adding more factors involved in the multiplication of *Salmonella* in other food sources (e.g. pork) and their linkage with other fundamental variables (vapour pressure, UV-light, humidity, etc.) will further improve our predictions at higher resolution. Understanding how salmonellosis is conditional on environment will be useful for practical public health applications, such as predicting the risk under different climate scenarios.

Funding: This study is funded by One Health EJP “EnvDis”

[P058] THE FOODCHAIN-LAB WEB APPLICATION AS INTEGRATIVE TOOL TO TRACE FOOD ALONG COMPLEX GLOBAL FOOD SUPPLY CHAINS IN FOODBORNE CRISES

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Aim: The free and open-source FoodChain-Lab web application (FCL Web) helps tracing food items along complex supply chains during food-related incidents. In the context of OHEJP COHESIVE, FCL Web will unite several tracing-related software projects in one modular platform following the One Health approach.

Methods: Agile software development.

Results: FCL Web offers an interactive analysis module, a reporting module and a synchronization module with the FCL desktop version. It is available at https://fcl-portal.bfr.berlin/. The interactive analysis module developed in a project with EFSA includes the automated visualisation of supply chain networks and simulations of scenarios such as cross contamination or geographic clustering and is now integrated in FCL Web. A first demonstrator of a reporting module was integrated in FCL Web as well. It displays tracing, sample and case information in a format which is suitable for publishing the results of tracing analyses in outbreak reports. A pilot version of a web-based tracing data collection mask was developed in a national project and will be integrated in FCL Web soon. It improves data quality by offering a guided and structured data assessment with access to curated data. Its multi-language design allows for potential European-wide use. In the future, more modules, e.g. to analyse genome sequencing data in the context of tracing are planned for FCL Web.

Conclusions: In times of complex globalised supply chains, powerful integrative e-tools are needed to efficiently solve foodborne crises. FCL Web is such a tool and unifies several tracing-relevant functionalities in one platform.

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[P059] ANALYSES OF ENVIRONMENTAL FACTORS DRIVING THE OCCURRENCE OF ANTIMICROBIAL RESISTANCE IN GERMAN WILD BOAR (SUS SCROFA): A GENERIC DATA PIPELINE APPROACH

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Aim: Antimicrobial resistant bacteria (AMRB) are a major public health threat, hence understanding environmental factors promoting the occurrence of AMRB in wildlife is paramount for One Health. Therefore, we analyzed data of the German National Zoonosis Monitoring Program 2016 using spatial statistics to identify decisive anthropogenic and ambient factors (livestock density, climatic variables, human density) based on *a priori* hypotheses of human-wildlife interfaces.

Methods: A generic data pipeline was developed in R 4.0.3. With this pipeline the results of 548 hunted wild boar (*Sus scrofa L.*) sampled and tested on ESBL/AmpC producing *Escherichia coli (E.coli)* were analyzed with binomial generalized linear mixed models (GLMM). Data of anthropogenic and ambient factors were extracted for each municipality and aggregated as median values. We further, clustered all sampling sites according to their geographic density and used them as random effect. Furthermore, 25 positive samples were spatially analyzed for their resistance determinants (RD).

Results: The GLMM indicated a significant positive influence of the livestock density on the occurrence of ESBL/AmpC producing *E. coli*. The RD support these findings, and further indicate human related resistances. Therefore, we compared the RD with data of communal sewage plants, which showed an association as well.

Conclusions: The results support the hypotheses that there is an association between AMRB in wild boar and certain anthropogenic factors. We further created a data integration pipeline to spatially analyze zoonoses monitoring data with anthropogenic and ambient factors. This pipeline can be used in the future also for other disease monitoring and disease surveillance activities.

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

[P060] DEVELOPMENT OF AN EX-VIVO INFECTIVITY ASSAY FOR HEPATITIS E VIRUS

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Aim: Pig products have been identified as the main infection route of Hepatitis E virus (HEV) infection in humans. Biosecurity measurements may be applied to reduce HEV in pig farms. To this date HEV detection relies on RT- PCR and because culture methods are lacking, assessment of HEV infectivity is hardly tried. Our aim is to develop a broadly applicable, feasible and sensitive culture system which can be applied for the detection of infectious HEV in swine faeces, environmental materials and food products.

Methods: Fresh liver tissue, obtained from a young piglet was perfused with collagenase IV. Liver cells were isolated and cultured, and subsequently hepatocytes were selected. As soon as these hepatocytes were growing confluent, they were inoculated with HEV positive samples. After incubation for 1.5 hours the inoculate was removed and the cells were washed prior to adding 2ml of the growth medium. The medium was refreshed for about 50% each 2nd or 3rd days during 7 days. Almost each day, from D0 to D7, culture medium was sampled and tested by real time rtPCR.

Results: A week after infection we observed a decreace of the Ct value of 8-10 units in the supernatants, and a decrease of 5-8 units in the cell fractions.

Conclusions: Based on the decreasing Ct values it can be concluded that there is virus replication. Further study to confirm our observations by EM or antigen detection, and to reproduce the results with different samples are ongoing.

Funding: This project is partially funded by OHEJP

[P061] DEVELOPMENT OF A WHOLE GENOME SEQUENCE METHOD FOR HEV

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Aim: Hepatitis E virus (HEV) genotype 3 is a zoonotic virus. HEV has a relatively high genetic diversity and its classification can best be done by whole genome sequencing. So far, to obtain the virus complete sequence a high concentration of HEV is needed. Our aim is to develop a sample processing protocol for recovery of enough RNA from HEV positive target samples of different origin and concentrations.

Methods: We want to be able to generate a complete HEV sequence from samples of different origin. To get this done it is important to improve the pre-processing of target samples and to optimise the enrichment of the RNA. Therefore, different DNA/RNA depletion treatments were explored to decrease the amount of host and bacterial (m)RNA/DNA. After an optimised RNA isolation, a full genome can be achieved by enrichment using the Secap protocol from roche using the virocap probeset or a HEV specific multiple rt-pcr genome amplification. Both methods will be further developed and compared regarding efficiency.

Results: We succeeded in reducing the amount of host and bacterial (m)RNA/DNA using an overnight benzonase pre-treatment. This ViroCap enrichment method enabled us to generate whole genome sequences up to an Ct value of 30 in faecal and liver samples. Genome amplification with multiple rt pcr is still under development.

Conclusions: We are able to generate whole genome sequences up to a Ct value of 30. The enrichment methodes are still under development. Comparison studies using different processing methods are ongoing.

Funding: This project is partially funded by OHEJP

[P062] POTENTIAL ROLE OF LIVESTOCK-ASSOCIATED ENTEROBACTER SPP. FROM PROCESS- AND WASTEWATER FROM GERMAN PIG AND POULTRY SLAUGHTERHOUSES AS A SOURCE FOR THE MOBILE COLISTIN RESISTANCE GENE MCR-9

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Aim: Recent reports highlight the emergence of clinical *Enterobacter* spp. isolates carrying carbapenem resistance genes in combination with the mobile colistin resistance gene *mcr*-9. This study gives insights into the phenotypic properties, genetics and transmission of *mcr*-9-carrying plasmids from *Enterobacter* spp. of environmental origin and their relationship to plasmids of clinically relevant isolates.

Methods: Out of 63 *Enterobacter* spp. recovered from process- and wastewater from pig and poultry slaughterhouses, 36 exhibited a colistin minimal inhibitory concentration of >2 mg/L. Those carrying mobile colistin resistance gene *mcr*-9 were further subjected to detailed phenotypic and genotypic investigations. The genetic basis of the *mcr*-9 resistance was deduced from whole-genome sequencing data and bioinformatics analysis. Transmission studies were conducted to assess the impact of *mcr*-9-plasmid for the development of colistin resistance.

Results: Phylogenetic analysis revealed that the three identified *mcr-9*-carrying isolates (two *E. roggenkampii* and one *E. kobeii*) could be assigned to two different clonal types based on their XbaI-macrorestriction profiles as well as based on SNP-trees. For each isolate, *mcr-9* was found to be located on a mobile genetic element (MGE). Both clonal types exhibit MGE, which substantially differ in their organization and composition. Up to now, the elements were assessed to be not transmissible by *in vitro* filter mating studies.

Conclusions: Wastewater might have an impact on the dissemination of *Enterobacter* spp. carrying *mcr*-9-associated mobile genetic elements. This may represent a hotspot for the dissemination of clinically relevant antimicrobial resistance to colistin.

[P063] IMPACT OF SEAFOOD IMPORTS FOR THE SPREAD OF ANTIMICROBIAL RESISTANT VIBRIO PARAHAEMOLYTICUS IN GERMANY: SURVEILLANCE, TYPING AND CHARACTERIZATION OF MULTIDRUG-RESISTANT ISOLATES

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Aim: In general, multidrug-resistant (MDR) *Vibrio parahaemolyticus* are increasingly reported from imported fish/seafood intended for distribution on the human food market. As fish/seafood represents a common reservoir for *V. parahaemolyticus*, the consumption of contaminated/undercooked food products may pose a risk for gastrointestinal infections in humans. The global commodity flow was determined as a main source for MDR isolates carrying resistances against highly and critically important antimicrobials.

Methods: Antimicrobial susceptibility testing (AST) of *V. parahaemolyticus* isolates was conducted by broth microdilution according to CLSI. Minimal inhibitory concentration (MIC) was interpreted according to the ECOFFs of EUCAST and MDR isolates were further subjected to *in-depth* characterization by S1-PFGE, *in vitro*-filter-mating studies and *short-read* whole-genome sequencing/bioinformatics analysis.

Results: AST of *V. parahaemolyticus* recovered between 2016 and 2018 revealed eleven ESBL- and one carbapenemase-producing isolates. XbaI-PFGE and sequence analysis showed a broad phylogenetic diversity of the isolates, different sequence types and resistance determinants (i.e. $bla_{CTX\text{-}M}$, bla_{CMY}). The majority of the MDR isolates have been shown to carry acquired ESBL-/carbapenem resistance determinants, of which some are located on mobile genetic elements (plasmids). Information on the transmissibility and genetic composition of the individual AMR carrying plasmids will be presented in detail.

Conclusions: While the number of MDR *Vibrio* from imported fish/seafood of South-East Asia increases, the question on the safety of food products from this region arise. Our findings show that antibiotic resistance monitoring for fish/seafood is needed to prevent the spread of MDR isolates in Germany and Europe.

[P064] UNDERSTANDING THE DEVELOPMENT OF FLUOROQUINOLONE RESISTANCE IN CAMPYLOBACTER OVER TIME

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Aim: *Campylobacter* is a common gastrointestinal pathogen, which has gained resistance to fluoroquinolone antimicrobials (FQ), regarded as critically important by the World Health Organisation. This project aims to identify temporal trends in the development and diversity of FQ resistance in *Campylobacter* sourced from different farming methods and at various stages of UK poultry production.

Methods: Phenotypic, genomic and epidemiological meta-data from isolates were collated from UK national surveillance studies from 1995 to 2020. Sequencing data of *Campylobacter* were analysed to determine genotypes associated with FQ resistance and identify resistance trends in relation to methods of production. We also investigated the association of multilocus sequence type (MLST) complexes with the trends in FQ resistance.

Results: The longitudinal dataset included in this study confirms a year-on-year increase in FQ resistant *Campylobacter*. The dominant mechanism of FQ resistance was found to be a threonine to isoleucine mutation at position 86 in the gyrase A gene. Initial analysis indicates a relationship between FQ resistance and both production methods and clonal complex.

Conclusions: Fluoroquinolone resistance in *Campylobacter* is a growing problem, with certain clonal complexes being strongly associated with this resistance. Some clonal complexes have been reported in human clinical cases which suggests the ability to persist throughout the food chain. Further control measures are needed to prevent wider dissemination of resistant strains.

[P065] RAPID LAMP DETECTION OF KEY AMR TARGETS FOR USE AS BED-SIDE AND/OR PEN-SIDE DIAGNOSTICS

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Introduction: Antimicrobial resistance (AMR) is a global issue affecting both human and animal health. The over-use and miss-use of antibiotics is the main driver of AMR, and therefore better diagnostics and interventions are urgently required. Rapid detection of AMR targets is crucial for identifying appropriate treatments, monitoring outbreaks and preventing further spread of AMR. Loop-mediated isothermal amplification (LAMP) is a rapid diagnostic platform for the detection of nucleic acid targets without the need for thermal-based machines.

Aim: This study aimed to develop a panel of LAMP assays to rapidly detect AMR bed-side and/or pen-side. LAMP primers were designed to specifically target plasmid-mediated colistin resistance (mcr-1), KPC-mediated carbapenem resistance (KPC) and oxacillin-hydrolysing β-lactamases (OXA-48 and OXA-23 genes).

Methods: Nucleotide sequences of each selected target and all representative alleles were used in multiple sequence alignment using Mega-X software to identify conserved sequences. Conserved sequences were selected to design LAMP primers using LAMP designer software and Primer Explorer.

Results: Mcr-1, KPC, OXA-48 and OXA-23 LAMP assays were tested and optimised to detect the targeted conserved sequences. All LAMP assays successfully detected the target genes within less than 5 min. The LAMP assays demonstrated a detection limit of approximately 1 pg and 10 pg DNA using fluorescence and colorimetric detection, respectively.

Conclusions: All LAMP targets were rapidly amplified with excellent sensitivity and specificity against all tested Gram-positive and -negative strains. Future studies will focus on assays' validation and implementation in the field as bed-side and/or pen-side diagnostics to facilitate the rapid detection of AMR.

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[P066] GENOMIC TAXONOMY OF KLEBSIELLA PNEUMONIAE STRAINS: APPLICATION OF CGMLST AND CGLINCODES

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Aim: Classification and naming of sublineages within bacterial species are largely lacking, which confuses communication on bacterial strain subtypes. Whole genome sequence-based microbial strain typing methods (cgMLST, HierCC, SNPadress) are considered promising tools to address this challenge.

Methods - Results: Here we propose a broadly applicable genome classification and nomenclature approach for bacterial strains, using as model the prominent public health pathogen Klebsiella pneumoniae. Phylogenetic and clustering analyses of >7,000 genome assemblies captured the population structure discontinuities, which were used as a guide to determine 10 infra-specific classification thresholds. We devised both a single linkage-based hierarchical clustering classification (HierCC) and stable proximity-based genome codes (cgLINcodes). Human-readable genomic sublineages and clonal groups identifiers were attributed by maximizing inheritance from the widely used 7-gene multilocus sequence typing (MLST) nomenclature.

Conclusions: The proposed strain taxonomy system combines phylogenetic-rich automated barcodes and human-readable identifiers, which will facilitate interoperability with other systems and global communication among microbiology, epidemiology and public health actors. Our species-specific operational approach for a unified genomic taxonomy of microbial strains is broadly applicable to other species of microbial pathogens and should facilitate collective understanding of epidemiology, emergence and microevolution of microbial pathogens.

[P067] BIOSECURITY MEASURES REDUCING SALMONELLA SSP. AND HEPATITIS E VIRUS PREVALENCE IN PIG FARMS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Aim: The study objective was to identify biosecurity measures (BSMs) that are relatively easy to implement and effectively reduce the prevalence of Salmonella or hepatitis E virus (HEV) in pig farms.

Methods: Scientific literature obtained from the Web of Science and PubMed databases was systematically reviewed. The extracted effects were estimated from raw data as odds ratios and their confidence intervals (95% CI). If more than one effect estimate was identified, the estimates were summarized applying meta-analytical fixed-effects models without any moderators. Weighted estimation with inverse-variance weights was applied.

Results: We identified 26 articles reporting 66 BSMs to reduce *Salmonella* and 4 articles including 15 BSMs to reduce HEV prevalence in pig farms. For *Salmonella*, animal movement to cleaned and disinfected finishing units, continuous flushing of gutters, presence of a lavatory at the farm, contact of < 3 humans per day with finisher pigs, and disinfection of farrowing and post-weaning pens were the five most effective BSMs. The use of a shower before entering the farm buildings, a sanitary ford at the farm entry, separate farm clothes, a perimeter fence around the farm, and maximally one foreign source of weaned pigs were the five most effective BSMs to reduce HEV.

Conclusions: These results demonstrate different key areas for animal management and hygiene strategies in biosecurity for pig farms. This study will provide information on the effectiveness of primary and secondary BSMs to reduce *Salmonella* and HEV prevalence in pig farms and support farm managers and veterinarians to refine biosecurity strategies.

[P068] MAPPING THE FOOD CHAIN TO IDENTIFY NEW OPPORTUNITIES FOR FOODBORNE ZOONOSIS SURVEILLANCE

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Aim: Data driven surveillance, often based on secondary data collected for other purposes than surveillance, is expected to be more cost effective than traditional surveillance. From a "One Health" perspective, no surveillance system based on secondary data is currently operational across veterinary, medical and environmental sectors in the EU. Our aim was to identify secondary data useful for foodborne zoonosis surveillance by mapping the food chain.

Methods: First, a general flow chart of the food chain and its environment was drawn and information flows associated to the material flows were identified. Second, the data sources associated to the information flows were placed on the map to visualize their coverage of the food chain. For this step, a specific example was choosen about the surveillance of *Salmonella* in France, Norway, Sweden and the United Kingdom.

Results: Forty-nine data sources were identified as relevant for *Salmonella* surveillance along the food chain in the four countries: 28 were dedicated to animal health, 13 to public health, one in feed, one in environment sector and six covered at least two sectors. A wide diversity of data sources was observed, from operational surveillance systems producing daily alerts to isolated databases with no centralisation of data.

Conclusions: The general flow chart provided a convenient framework to identify existing data sources for inter-sectoral foodborne zoonosis surveillance. It made easy to pinpoint possible but yet unused data sources. Evaluation of data completeness, timeliness and accuracy would allow determining the relevance of those data sources for data driven surveillance.

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[P069] THE SIGNIFICANCE AND EXPECTED IMPACT OF THE JOINT RESEARCH PROJECTS OF THE ONE HEALTH EJP

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Aim: The overall objective of the One Health EJP is to create and consolidate a network of One Health partners in Europe. One major pathway in this process is to bring together researchers from animal health, public health and food safety institutes that are active in the domains of foodborne zoonoses, antimicrobial resistance and emerging threats. The present analysis aims at illustrating the significance of Joint Research Projects (JRP) and their expected impact on improving the preparedness of the consortium partners.

Methods: In the reports for 2020, project leaders of the 24 JRPs categorized the project deliverables according to their relevance for surveillance, i.e. the design of surveillance programmes, laboratory activities, risk assessment and interventions, following the integrative strategy matrix developed in the One Health EJP Strategic Research Agenda.

Results: All JRPs produced outcome that not only advance scientific knowledge, but that also have integrative aspects that feed into the different parts of the surveillance process.

Conclusions: The results exemplify how integrative aspects of JRPs add to the One Health EJP principal objective of intensifying One Health collaboration among partners. The strengthening of the preparedness is best accomplished in peacetime, by learning about each other's capabilities and capacities, by developing complementary activities and by building trust among partners. Working together in relevant research projects will reinforce cooperation and collaboration among sectors, as aimed for in the One Health EJP.

References:
One Health EJP projects https://onehealthejp.eu/projects/

One Health EJP Strategic Research Agenda https://onehealthejp.eu/wp-content/uploads/2018/12/One-Health-EJP-Strategic-Research-Agenda.pdf

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[P070] HOW TO INCREASE THE SENSITIVITY OF CPE ISOLATION METHOD FROM CAECAL SAMPLES

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Aim: Carbapenem-resistant Enterobacteria are a severe threat to human health. In recent years, they were sporadically isolated from livestock. For the mandatory monitoring of carbapenemase-producing *E. coli* (CPE) in caecum in accordance with CID 2013/625/EU an isolation protocol is provided by the EURL-AR[1]. In some cases, the method failed to detect CPE with low MIC values for carbapenems. To increase the sensitivity, some modifications were investigated.

Methods: For isolation of CPE from caecum, the introduction of a second, selective enrichment step (LBL + 1 mg/L cefotaxim (CTX) and LBL + 0,125 mg/L meropenem (MEM) at 37 °C under microaerophilic conditions) and an additional Real-Time-PCR-screening was investigated. Moreover, we tested the use of alternative selective agars (McConkey agar (McC) +MEM and McC+MEM+CTX) instead of commercial agars. Both methods were performed on 54 blinded samples.

Results: The comparison showed that the sensitivity of the method is largely dependent on the agar used. By implementing the EURL-method in combination with the *in-house* selective agar, a sensitivity and specificity of 100 % was achieved. The use of a commercial agar decreased the sensitivity to ~75 %. The second enrichment showed no substantial influence on isolation but increased the gene detection by PCR to 100 %. However, this step may improve the isolation by reducing the accompanying flora and thus, increasing the detection of suspicious colonies.

Conclusions: The use of the *in-house* agar increases the isolation of CPE from caecal samples. Excessive growth of accompanying flora can be reduced with a second, selective enrichment step.

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https://www.eurl-ar.eu/protocols.aspx

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[P071] UNPRECEDENTED WHOLE GENOME SEQUENCING EFFORT REVEALS HIGHLY POLYMORPHIC REGIONS IN THE GENOME OF EUROPEAN TOXOPLASMA GONDII STRAINS

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Aim: *Toxoplasma gondii* is an important zoonotic protozoan parasite that has a clonal population structure consisting largely of three genotypes in Europe. The aim of this work was to identify and characterize polymorphic loci in the closely related strains circulating in Europe.

Methods: *T. gondii* field isolates were collected from different parts of Europe and genotyped using standard methods. A whole genome sequencing (WGS) analysis was conducted on more than 60 cell-cultured *T. gondii* isolates and highly polymorphic regions (HPRs) were identified in the genomes. These regions showed a considerable number of single nucleotide polymorphisms (SNPs). In a second step, the HPRs identified by WGS were confirmed by Sanger sequencing using novel primer pairs.

Results: A large number of HPRs relative to a reference genome were identified (n=19 with ≥ 20, n=37 with 15-19, and n=137 with 10-14 SNPs per 333 bp). For many of the regions, Sanger sequencing has confirmed the HPRs predicted by WGS analysis.

Conclusions: The results suggest the existence of an unexpected high number of HPR in European T. gondii isolates. This new knowledge advances our understanding of the genetic variation within this parasites that has an extremely wide host range in nature.

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[P072] TRICHINELLA SPP. IN KEY WILDLIFE HOST SPECIES IN ESTONIA

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Aim: We investigated *Trichinella* spp., which are important zoonotic parasites, in wildlife in Estonia. This presentation summarizes the results on *Trichinella* spp. in three key host species: wild boars (*Sus scrofa*), raccoon dogs (*Nyctereutes procyonoides*) and red foxes (*Vulpes vulpes*).

Methods: Sampling for the studies was done in 2007–2014. From wild boars, 470 meat juice samples were tested for anti-*Trichinella* IgG by ELISA and 52 also with western blot. Muscle samples (30,566 from wild boars, 113 from raccoon dogs, and 87 from red foxes) were tested by artificial digestion method. *Trichinella* species were determined by multiplex-PCR.

Results: Based on digestion results, 0.9% of wild boars were found infected with *Trichinella* spp., while 42.1% were positive by ELISA, and a western blot confirmed estimate of seroprevalence was 17.4%. *Trichinella* spp. infection prevalence was 57.5% in raccoon dogs and 69.0% in red foxes. *Trichinella britovi* was detected in 0.7% of the wild boars, 24.8% of the raccoon dogs and 35.6% of the red foxes, and *Trichinella nativa* in 0.1% of the wild boars, 31.9% of the raccoon dogs and 31.0% of the red foxes. *Trichinella spiralis* was found in 0.03% of the wild boars, and *Trichinella pseudospiralis* in 0.02% of the wild boars.

Conclusions: The findings confirmed the presence of high infection pressure of *Trichinella* in wildlife in Estonia. Several wildlife species are hunted in the country for human consumption, and spillover to domestic animals is also of concern. Meat control is continuously important to avoid *Trichinella* infections in humans.

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[P073] TOXOSOURCES-PROJECT: WHAT IS THE RELATIVE IMPORTANCE OF THE DIFFERENT SOURCES OF TOXOPLASMA GONDII INFECTION IN EUROPE?

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Aim: *Toxoplasma gondii* is a foodborne parasite that causes a high disease burden in Europe. The infection can be acquired by ingesting oocysts (environmental pathway) or tissue cysts (meatborne pathway). TOXOSOURCES-project aims to investigate the relative importance of these transmission routes to the infections in humans, which is still unknown, partly due to lack of appropriate methods.

Methods: TOXOSOURCES is a Joint Research Project of the One Health EJP that focuses on *T. gondii* at the interface between humans, animals, food, and the environment. A multidisciplinary approach using modelling, multicentre field studies, experimental designs, and novel and improved methods is developed.

Results: During the first year of TOXOSOURCES, the collection of data and building of a quantitative microbiological risk assessment model were started. A questionnaire was developed for collecting food consumption data across Europe. The optimal molecular method for *T. gondii* oocyst detection in fresh produce for a multicentre survey was selected. Bioinformatic selection of promising protein candidates for a serology method able to distinguish environmental from meatborne infections was finalized. The retrieval of *T. gondii* isolates and DNAs from across Europe for Whole Genome Sequencing was successful.

Conclusions: The outcomes of TOXOSOURCES will include quantitative estimates of the contribution of the main sources and transmission routes to *T. gondii* infections based on improved source attribution models, and new data filling the knowledge gap regarding the role of ready-to-eat fresh produce. Moreover, approaches for detecting infections caused by oocysts using serology and for tracing infection sources in outbreaks are developed.

Funding: TOXOSOURCES project is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

References:
TOXOSOURCES project website https://onehealthejp.eu/jrp-toxosources/

[P074] ZOONOTIC RISK OF IMPORTED DOGS IN FINLAND

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Aim: Importing dogs, especially shelter dogs, has become more popular in Finland. As the situation of zoonotic diseases in their countries of origin differs from the situation in Finland, we studied the zoonotic risk related to dog import.

Methods: Dogs, which had been imported within one month were sampled. Rabies, *Brucella canis*, *Leishmania infantum* and *Dirofilaria immitis* antibodies and *Dirofilaria repens* were studied from blood samples. Presence of antimicrobial resistant bacteria was studied from swab samples and *Echinococcus multilocularis* from fecal samples. Furthermore, rabies antibodies were tested from blood samples of 90 dogs at the border. Expert interviews and a questionnaire study for veterinarians were conducted.

Results: The studied dogs were imported from 14 countries, with the majority arriving from Russia, Romania, or Spain. The rabies antibodies were undetectable in samples of 28% (48/170) of the dogs originating from five countries. ESBL- or AmpC-positive *Escherichia coli* was detected in 30% (25/85) of the dogs from five countries. Two dogs were *B. canis* -positive. None of the dogs was infected with *E. multilocularis*, *D. immitis* or *D. repens*. Four dogs had borderline results for *L. infantum*.

Conclusions: There is a potential for sporadic human or animal infections via imported dogs. The amount of dogs imported without proper vaccination status against rabies was alarmingly high. The carriage of antimicrobial resistant bacteria was also common. The country of origin and the holding conditions there affect the disease risk caused by imported dogs.

[P075] "CASSETTOMICS": METAGENOMICS OF MOBILE INTEGRONS GENE CASSETTES FROM EFFLUENTS

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Aim: Class 1 integrons are bacterial genetic elements widely involved in the spread of antibiotic resistance[1]. They are present in high quantities in hospital effluents[2] and considered as markers of anthropic pollution in the environment They can recruit, express and disseminate antibiotic resistance genes embedded within gene cassettes (GCs). In silico analysis revealed 427 distinct GCs, 175 encoding resistance to nearly all antibiotic families.

Our objective was to extensively characterize the pool of GCs of hospital, animal and environmental origins.

Methods: Total DNA was extracted from European hospital (n=10), slaughterhouses (n=8), urban (n=2) effluents, 2 wastewater treatment plants influents and from 2 rivers. GCs were amplified by end-point PCR and PCR products were sequenced using Next Generation Sequencing with the Ion Proton® system. Reads were mapped against the 427 known GCs. Unmapped reads were assembled as contigs. Contigs longer than 500bp were screened for the presence of GCs using the IntegronFinder (IF) software[3]. IF-positive contigs were manually analysed to identify novel GCs. All reads were then mapped to an updated list of GCs enriched with these novel GCs.

Results: 357 novel GCs were identified. Raw reads mapping revealed a high proportion of aminoglycosides and β-lactams resistance GCs in all hospital effluents. The mean number of GCs and the diversity of the GC pool were similar in hospital and slaughterhouse effluents but higher in environmental effluents. Principal components analysis could discriminate the GC pools according to their origin.

Conclusions: This high-throughput NGS-based "cassettomic" approach allows to characterize the pool of GCs and discriminate effluents origins.

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[P076] HIGH DIVERSITY OF PLASMIDS CARRYING QNR RESISTANCE GENES IN FLUOROQUINOLONE RESISTANT ESCHERICHIA COLI ISOLATED IN GERMANY IN 2017

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Aim: The prevalence and diversity of *qnr*-carrying isolates among (fluoro-)quinolone (FQ)-resistant, commensal *E. coli* from the German annual antimicrobial resistance monitoring in livestock and food in 2017 was determined. The data will be used to understand mechanisms involved in FQ-resistance development and the diversity of mobile genetic elements associated with their spread.

Methods: A total of 3,409 *E. coli* isolates from the German National Reference Laboratory for Antimicrobial Resistance was investigated. Antimicrobial resistance testing was conducted by broth microdilution according to CLSI guidelines. MIC values for ciprofloxacin and nalidixic acid were evaluated using EUCAST epidemiological cut-off values. Isolates exhibiting resistances to FQ were subjected to *qnr*-PCR, *XbaI*-/S1-PFGE, whole genome sequencing (WGS) and bioinformatics analysis.

Results: Overall, 504 isolates were classified as quinolone-resistant, while only 107 of them harbored a *qnr* gene. *XbaI*-macrorestriction demonstrated a high heterogeneity, letting us assume that no predominant *E. coli* lineages are associated with FQ resistance development in Germany. S1-PFGE analysis showed a variety of extrachromosomal elements of various sizes. Short-read WGS revealed *qnr* to be associated with *bla* as well as with the disinfectant *qacEΔ1*. The most abundant plasmid replicon type among our identified *qnr* reference plasmids were IncN, IncY and IncX1-IncX3. Further, we frequently detected chromosomal point mutations leading to an altered susceptibility against FQs.

Conclusions: *qnr* genes were shown to be carried by multiple plasmid types and in frequent association with other resistance genes. This diversity of *qnr* plasmids in combination with their multi-resistance demonstrates the risk associated with *qnr* positive *E. coli*.

[P077] EVOLVING AT THE SPEED OF RISK

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Aim: Diverging perspectives on what constitutes risk concerning emerging threats from (re)emerging or antimicrobial-resistant pathogens (REIDAMRs) exist across scientific and management disciplines. In this integrative effort we identify risk questions, and assess theoretical lead time from emergence to risk management decisions in the EU and Norway for different REIDAMRs depending on risk question and corresponding parameters. In the process we attempt to identify fundamental knowledge gaps faced by management with regards to transmission and virulence trajectories, and in how to summarize complex health messages to the public. We present status for this work in progress.

Methods: Literature and media studies complemented with models of spread for different disease parameters using the GLEaMviz [1] tool coupled with EID and AMR hotspot analyses [2,3] and with virulence evolution models building on and integrating existing work.

Results: Risk questions depend on discipline and include: (i) surveillance and detection; (ii) patient outcomes, (iii) individual probability of infection; (iv) danger of emergence, selection and spread of REIDAMRs or genetic elements, and; (v) indirect effects through interactions with other microbes and hosts. Lead times from emergence to awareness vary greatly and depend on socioeconomic as well as biological factors. A major unknown factor is our limited ability to predict pathogen evolutionary trajectories.

Conclusions: Some trends that can help allocate surveillance resources and suggest interventions can be identified to increase warning time and reduce several risk aspects. Some possible key knowledge holes and approaches to evolutionary research are described. Public communication plans and summaries are under development.

[P078] PROTEOMIC AND BIOINFORMATIC INVESTIGATIONS OF HEAT-TREATED ANISAKIS SIMPLEX THIRD-STAGE LARVAE

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Aim: The aim of the study was proteomic and computational investigations of heat-treated at 121 °C for 60 min proteins of *Anisakis simplex* L3 larvae.

Methods: SDS-PAGE and Western Blot analyses were performed to investigate the influence of high temperature on the *Anisakis* antigen. The autoclaved *A. simplex* antigens after standard sample processing were subjected to liquid chromatographyt mass spectrometry (LC-MS/MS) analysis. Identified peptides/proteins were analyzed in silico using different computational tools.

Results: A total of 470 proteins including allergens—Ani s 1, Ani s 2, Ani s 3, Ani s 4, Ani s 5—and 13 potential allergens were found in all three biological replicates of shotgun LC-MS/MS analysis. Potential allergens were mainly homologs of *Anisakis* spp., *Ascaris* spp., and Acari allergens. Ani s 2, Ani s 3, Ani s 5, and three possible allergens were found among the top 25 most abundant proteins. The computational analysis allowed us to detect epitopes and putative epitopes of allergens, assign protein families, and domains as well as to annotate the localization of proteins. The predicted 3D models of proteins revealed similarities between potential allergens and homologous allergens. Despite the partial degradation of heated *A. simplex* antigens, their immunoreactivity with anti-*A. simplex* IgG antibodies was confirmed using a Western blot.

Conclusions: Identified epitopes of allergenic peptides highlighted that the occurrence of *Anisakis* proteins in thermally processed fish products could be a potential allergic hazard. Further studies are necessary to confirm the IgE immunoreactivity and thermostability of identified proteins.

[P079] NEW INSIGHTS INTO THE PROTEOMES OF ANISAKIS SIMPLEX, PSEUDOTERRANOVA DECIPIENS, AND CONTRACAECUM OSCULATUM

Maciej Kochanowski,[1], *Mirosław Różycki,*[2], *Joanna Dabrowska,*[2], *Jacek Karamon,*[2], *Jacek Sroka,*[2], *Weronika Korpysa-Dzirba,*[2], *Ewelina Antolak,*[2], *Aneta Bełcik,*[2], *Tomasz Cencek,*[2]

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Aim: We aimed to provide more insight into Anisakidae proteomes by comparative proteomic investigation of *Anisakis simplex*, *Pseudoterranva decipiens*, and *Contracaecum osculatum* L3 larvae.

Methods: SDS-PAGE and IgG-Western Blot analyses of parasite extracts were performed. Parasite extracts after standard sample processing were subjected to liquid chromatography mass spectrometry (LC-MS/MS) analysis. Detected proteins were investigated using different bioinformatic tools.

Results: In total, 645, 397, and 261 proteins were detected in *A. simplex*, *P. decipiens*, and *C. osculatum* L3 larvae, respectively. Western Blot analysis confirmed the cross-reactivity of IgG anti-*A. simplex* antibodies with protein extracts from *P. decipiens* and *C. osculatum* L3 larvae. The identified proteins of the Anisakidae proteomes were characterized by label-free quantification and functional analysis, and proteins involved in many essential biological mechanisms, such as parasite survival, were identified. In the proteome of *A. simplex* 14 allergens were identified. Eight allergens were detected in *P. decipiens*, while in *C. osculatum* 4 allergens were found. Furthermore, 28 probable allergens were predicted in *A. simplex* and *P. decipiens*, whereas in *C. osculatum*, 25 possible allergens were identified. Among the putative allergens, heat shock proteins were most frequently detected, followed by paramyosin, peptidyl-prolyl cis-trans isomerase, enolase, and tropomyosin.

Conclusions: We provide a new proteomic data set that could be beneficial for the discovery of biomarkers or drug target candidates. Furthermore, our findings showed that in addition to *A. simplex*, *P. decipiens* and *C. osculatum* should also be considered as potential sources of allergens that could lead to IgE-mediated hypersensitivity.

Acknowledgement: I acknowledge the support of the Med-Vet-Net Association in the form of a Conference Grant, which enabled me to attend this conference.

[P080] HEPATITIS E IN HUMANS AND PIGS IN BULGARIA (REVIEW)

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Aim: The aim of the literature review is to summarize current data on the prevalence of Hepatitis E virus (HEV) in human and pig population in Bulgaria to 2021. This review is based on reported cases of prevalence of HEV in hospitalized humans and prevalence of HEV infection in different groups of pig population in Bulgaria.

Methods: The scientific papers and reviews, published in PubMed Databases and Bulgarian medical databases, were analyzed. Data on the prevalence of Hepatitis E virus in Bulgaria from January 1995 to December 2020 were summarized.

Results: During the period (1995-2020) it was a 16 paper about prevalence of HEV in human and pig population in Bulgaria. They have been reported 6247 blood serums from hospitalized patients and 1381 serum samples from pigs at different ages were tested. In total 923 samples (14.7 %) from the hospitalized patients were positive for HEV. AntiHEV-IgG were found in 820 specimens (88.8 %) and HEV-RNA was present in 103 of those (11.2 %). The first data for HEV in pigs in Bulgaria were published in 2017. In total 846 from 1381 pig serum samples (61.2 %) were positive for antiHev-IgG. Of these, 52 samples (6.1 %) were from wild boar.

Conclusions: The spread of HEV among the populations of humans and pigs in Bulgaria is worrying. The data from the literature review are insufficient to establish the relationship between the virus in humans and pigs in Bulgaria. The hepatitis E virus needs to be considered in the differential diagnosis of acute human hepatitis. The question of its usefulness as an indicator of pork food safety is also debatable. The development of a unified harmonized strategy for monitoring and control of this zoonotic agent is imperative.

[P081] GES BETA-LACTAMASE GENES LOCATED ON VARIOUS PLASMIDS IN GRAM-NEGATIVE BACTERIA FROM WASTEWATERS

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Aim: Recently, GES-type extended-spectrum beta-lactamases have been documented more frequently from hospital setting as well as environment including variants with carbapenem-hydrolysing activity. This study was focused on detailed molecular characterization of GES-producing Gram-negative bacteria from municipal and hospital wastewater in the city of Brno, Czech Republic.

Methods: From a total of 120 GES beta-lactamase producing Gram-negative bacteria originating from hospital wastewater and inflow/outflow of municipal wastewater treatment plant, sixty-eight representative isolates were chosen based on their clonal diversity. The isolates were tested for susceptibility to 21 antimicrobials and carbapenemase production, and subjected to shotgun sequencing on Illumina platform. Additionally, the long-read sequencing (MinION) was performed to obtain closed structures of GES-borne plasmids. The transferability of bla_{GES} was tested by conjugation.

Results: Predominance of bla_{GES-1} (51%, no carbapenem-hydrolyzing activity) and bla_{GES-5} (49%, carbapenem-hydrolyzing activity) variants were observed among the isolates (n=68) and mainly carried on ColE2-like and IncQ2 plasmids. Other resistance genes for different antimicrobial groups including aminoglycosides, fosfomycin, sulphonamides, etc. were also detected. The conjugation transfer of bla_{GES} was successful only in 18% of the isolates and was mainly associated with ColE2-like plasmids detected across the various genera (*Enterobacter*, *Escherichia* and *Citrobacter*). The majority of ColE2-like plasmids also harboured Δ*intI1, aacA4* and *aadA10*-like resistance genes. Plasmid pEcl-355771cz with similar structure was detected previously from a clinical isolate in the Czech Republic.

Conclusions: Multidrug-resistant GES-producing bacteria were particularly associated with ColE2-like and IncQ2 plasmids. This is increasingly worrisome as the treated water is flowing into the environment.

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[P082] THE WHY AND HOW OF DNA SEQUENCING FOR ONE HEALTH – SETTING UP A BIOINFORMATICS PLATFORM

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Aim: DNA sequencing analyses can be used to characterize pathogens with regards to for instance antimicrobial resistance, virulence and similarity to other isolates. This information can be used for surveillance, and for outbreak detection and investigation. The aim of this study was to investigate which analyses would prove useful for which purposes, and also what consequences that would have for the setup of the IT systems used for analyses.

Methods: Performing sequencing analyses is often technically challenging, and the results can be difficult to interpret. Thus, web frameworks have been created to ease analyses. The methods in one of these, the IRIDA (https://www.irida.ca/) system, were evaluated with regards to usefulness in various scenarios. These scenarios were culled from day-to-day situations encountered by people at the Norwegian Veterinary Institute.

Results: In this work, we are establishing SOPs for various scenarios. These range from solving an outbreak, tracking “house strains”, to surveillance of genomic features such as virulence. In addition, considerations regarding the need for sharing sensitive and/or confidential metadata is discussed. The different scenarios and the results appropriate for each of them are presented.

Conclusions: Considering what the results will be used for is important when setting up a platform for sequencing analyses. The results have to be fit for purpose, and that has consequences for the setup and for how users can interact with the contents of the system.
This project received funding from the European Union’s Horizon 2020 research and innovation programme under Grant Agreement No 773830.

[P083] USE OF MULTI-LOCUS VARIABLE-NUMBER TANDEM REPEAT ANALYSIS (MLVA) TO IDENTIFY THE SOURCE OF CONTAMINATION OF A CATTLE BOTULISM OUTBREAK

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Aim: In 2018, a botulism outbreak occurred on a mixed farm with cattle and poultry productions, resulting in the death of 92 cows. A cross contamination between poultry and cattle was highly suspected based on the chronology of the events and the analysis of samples collected in both productions, where 52 were positive. A contamination from positive poultry manure to cattle through the tractor bucket identified was of particular interest. Further investigations in the hatchery delivering chicks to the farm allowed the detection of 6 other positive samples. The aim of this study was to compare a selection of these positive samples using a novel Multi-Locus Variable Number of Tandem Repeats (VNTR) analysis (MLVA) method to determine if poultry production was indeed the source of contamination of this outbreak.

Methods: Thirteen samples positive for *Clostridium botulinum* were selected among cattle, poultry productions and hatchery for MLVA analysis using nine PCR (one per VNTR). DNA extracts were directly used without any isolation step, allowing quick typing of the samples. The number of repeats for each VNTR were imported in BioNumerics software to build a dendrogram.

Results: MLVA analysis revealed that the same profile was detected in cattle (ruminal content from an affected cow), asymptomatic poultry (manure, cloacal swabs, environmental swabs) as well as in the hatchery (environmental swabs), confirming that poultry is the initial source of contamination.

Conclusions: This study shows that MLVA is a very powerful tool for *C. botulinum* tracking and epidemiological investigations in the context of animal botulism outbreaks.

[P084] DIFFERENTIAL SUSCEPTIBILITY OF SARS-COV-2 IN ANIMALS: EVIDENCE OF ACE2 DISTRIBUTION IN TISSUES OF COMPANION ANIMALS, LIVESTOCK AND WILDLIFE BY IMMUNOHISTOCHEMICAL CHARACTERISATION

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Aim: Angiotensin converting enzyme 2 (ACE2) is an important cell membrane protein that mediates the binding of several coronaviruses, most notably SARS coronaviruses. Although SARS-CoV-2 infection is mainly confined to humans, there have been numerous incidents of spillback into captive and companion animals where they are in close proximity with humans. In these cases, SARS-CoV-2 has been detected in the respiratory and gastrointestinal systems, however, infection has resulted in variable presentation of clinical disease. Consequently, the spatial distribution of ACE2 in animal tissues has not been well studied and this restricts our understanding of host species susceptibility.

Methods: This study investigates the distribution of ACE2 in histological sections derived from members of the *Bovidae, Suidae, Equidae, Felidae, Canidae, Mustelidae and Hominidae*. To develop robust ACE2 immunohistochemistry (IHC) detection, antibody reactivity is characterised using formalin-fixed paraffin-embedded (FFPE) BHK-21 cells expressing species-specific ACE2. This is followed by IHC characterisation of the receptor distribution in the lung and intestine as well as nasal mucosa of a subsample of species.

Results: Comparison of the mink and ferret respiratory tract showed substantial difference in ACE2 expression between the upper and lower respiratory tract of the two species which is reflected upon in disease susceptibility. Furthermore, ACE2 is present on the bronchiolar epithelium of sheep, cattle, European badger, and several species of large felids. In the intestine, ACE2 is distributed ubiquitously across all taxa with predominant expression in the villus.

Conclusions: The results provide evidence of cellular and organ-specific ACE2 expression which will enhance our understanding of species susceptibility, experimental pathogenesis and strategies for virus surveillance.

[P085] WHOLE GENOME SEQUENCING OF LINEZOLID-RESISTANT STAPHYLOCOCCUS AUREUS ST398 FROM HEALTHY PIGS IN PORTUGAL

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Aim: Livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA) ST398 is associated with healthy pigs and has been found colonizing or causing infections in humans. Here, we report the genomic characterization of three LA-MRSA isolates resistant to linezolid.

Methods: Whole genome sequencing by Illumina NovaSeq platform was performed in three linezolid-resistant isolates (MIC=8 µg/ml) recovered from pig nasal swabs. Trimmomatic, SPAdes, QUAST and Prokka were used for preprocessing of reads, *de novo* assembly, assembly statistics and genome annotation. Genome analyses were assessed using the Center for Genomic Epidemiology tools. Genetic platforms of *cfr* resistant gene were analyzed using Artemis, EasyFig and BLAST.

Results: All strains belonged to ST398 and t011 spa-type. The WGS predicted phenotype fully correlates with the multidrug resistant profile found in the susceptibility tests. Resistance genes encoding resistance to oxazolidinones, macrolides, lincosamides, streptogramins and florfenicol (*cfr*); to florfenicol (*fexA*); to β-lactams (*mecA* and *blaZ*) and tetracycline (*tetM* and *tetK*) were found. Furthermore, one isolate also carried *aadD* and two isolates harbored *dfrG* and *dfrK* genes, encoding resistance to aminoglycosides and trimethoprim, respectively. Several plasmids were identified. The *cfr* genes were flanked by IS*Sau9* and TnpR, and BLAST analysis revealed that were located at pSAM13-0401 plasmid.

Conclusions: To our knowledge, this is the first report of linezolid resistant LA-MRSA strains carrying the *cfr* gene in Portugal. The presence of plasmid-mediated *cfr*-positive LA-MRSA isolates may represent a risk of transmission to humans, particularly those in contact with livestock.

[P086] ESBL PLASMID TRANSFER BY HOST- AND COUNTRY- ASSOCIATED E. COLI IN AN IN VITRO MODEL OF THE CHICKEN CAECA

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Aim: To assess the impact of the simultaneous inoculation multiple *Escherichia coli* strains harbouring different ESBL plasmids in an *in vitro* model of the chicken caeca.

Methods: A continuous flow *in vitro* model of the chicken caeca was inoculated with a cocktail of 17 ESBL harbouring *E. coli* strains. These were associated with four different hosts and from four different countries. Samples from the vessels were plated onto culture media to enumerate the number of total bacteria, total *E. coli*, ceftiofur-resistant *E. coli* and the original cocktail strains (ceftiofur- and rifampicin-resistant). The fate of individual strains within the model was monitored using ORFan gene multiplex PCR assays. Finally, replica plating was used to identify any commensal *E. coli* that had acquired ESBL resistance genes.

Results: The cocktail strains were able to persist during the 72-hour experiment, although the total CFU/ml and number of individual strains decreased over time. The multiplex PCR assays showed that different strains were present in the vessels for varying lengths of time. No trans-conjugants were detected when 10^8 CFU/ml of the *E. coli* isolate cocktail was added to the model. When vessels were inoculated with a 10^{10} CFU/ml cocktail and potential trans-conjugants were isolated in samples taken at 48 and 72 hours post inoculation.

Conclusions: Here, we have shown that multiple ESBL-producing *E. coli* strains can persist within an *in vitro* model of the chicken caeca for 72 hours and some of these appear able to transfer their ESBL plasmid to the pre-existing commensal *E. coli*.

[P087] WHOLE GENOME SEQUENCING: EVALUATION OF VARIANT CALLING PIPELINES FOR THE STUDY OFANIMAL TUBERCULOSIS

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Aim: The aim of this study was to: 1) evaluate the availability of Whole Genome Sequencing (WGS) tools in the study of animal tuberculosis (aTB) and 2) provide evidence to animal health authorities regarding the possible implementation of these technologies in its eradication and surveillance programmes.

Methods: Four WGS pipelines (vSNP, bovTB, snipgenie and MTBseq) developed by animal and human health laboratories were evaluated in the context of their possible application by veterinary reference laboratories in the control and eradication of aTB. Their methodologies, tools, parameters and filters were compared in order to assess the existence of differences in their results and how they could affect conclusions drawn from them. Finally, their performance was evaluated using a simulated dataset of Illumina reads based on already published sequence data from a bovine TB high prevalence setting in the Republic of Ireland.

Results: The main differences between pipelines could be grouped into several categories: 1) variant calling method, 2) variant filters used, 3) type of output data for phylogenetic analysis and 4) pipeline modularity. All pipelines performed equally well when evaluating the simulated dataset and epidemiological conclusions did not vary significantly depending on the tool used.

Conclusions: As the use of WGS technologies in the study of aTB has increased in recent years, it is crucial to assess the availability of genomic tools. Under our simulation, current WGS tools offer equivalent performance, but more studies are needed to standardise the methodology in order to provide accurate and comparable results around the world.

[P088] MONITORING ANTIBIOTIC RESISTANCE GENE PROFILES IN HOSPITAL WASTEWATER – A PROOF- OF-CONCEPT STUDY USING RESISTAPP

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Aim: The aim of this study was to demonstrate proof-of-concept for ResistApp – a digital platform for antibiotic resistance monitoring in hospital wastewater. ResistApp combines culture-independent, high throughput gene quantification with automated data analysis to synthesise and visualise monitoring data in an interactive dashboard.

Methods: We studied two hospitals in Helsinki, Finland (HUS1 and HUS2). HUS1 used six times more antibiotic amounts than HUS2. We monitored wastewater from the two hospitals over nine weeks (weeks 25-33 in 2020) for a total of 216 antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), integrons, and taxonomy of bacteria, including bacteria causing hospital-acquired infections, and the 16S rRNA gene using high-throughput quantitative PCR (HT-qPCR). We analysed and visualised data from HT-qPCR using ResistApp.

Results: We detected a higher number of ARGs and MGEs at both hospitals in weeks 27-30 compared to other sampling weeks, with weeks 27-30 grouped separately from other sampling weeks by NMDS-analysis. *Bla*GES was the most abundant and prevalent carbapenem resistance gene in both hospitals throughout our sampling period. Our correlation analysis revealed a positive association between *bla*GES and MGEs in both hospitals. We also found more positive associations between carbapenem resistance genes and MGEs in HUS1 than HUS2 and a strong positive association between *bla*KPC and *Klebsiella pneumoniae* in HUS1 wastewater.

Conclusions: Monitoring hospital wastewater using ResistApp can capture the impact of antibiotic use on resistance profiles and the dynamics of these profiles. ResistApp can potentially be used as an early warning system to detect emerging outbreaks of resistant bacteria in hospitals.

Funding: The project was supported by Business Finland R&D Funding (Project No. 287/31/2020).

[P090] NOVEL PROBE-BASED REAL-TIME PCRS FOR DETECTION AND DIFFERENTIATION OF ECHINOCOCCUS SPECIES

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Aim: Infections with eggs of *Echinococcus* species can cause cystic or alveolar echinococcosis in intermediate host animals and humans. Cost-effective molecular assays with a high discriminative power are needed to investigate the dynamics, pathogenicity and infection routes of members of the genus Echinococcus.

Methods: COX1, COX3 and NAD5 primers discriminating between the species (according to post-amplification melting curves) were tested together with the respective probes in a TaqMan real-time PCR. For the analysis of analytical sensitivity and specificity of the real-time PCRs (SYBR-green and TaqMan), a panel of reference DNA samples from various *Echinococcus* spp. and *Taenia* spp. was used.

Results: We developed TaqMan real-time PCRs to target polymorphic regions in the mitochondrial genomes of members of the *E. granulosus sensu lato* complex and of *E. multilocularis*. In a single-step typing approach, we distinguished *Echinococus* species in six epidemiologically relevant subgroups. These were *E. granulosus sensu stricto* (G1, G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* strain G8, the *E. canadensis* cluster (G6-7, and G10) and *E. multilocularis*. The technique also allowed identification and differentiation of these species from *Taenia* spp. with samples isolated from cysts or faeces.

Conclusions: Single-step genotyping techniques for the molecular diagnosis of *Echinococcus* spp. by qPCRs may not only improve the diagnostic performance, but also our knowledge on the epidemiology of the parasites. They may thus help to control alveolar and cystic echinococcosis.

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[P091] WHOLE-GENOME SEQUENCING OF PHENOTYPICALLY CHARACTERIZED ISOLATES FROM VARIOUS SETTINGS

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Aim: Antibiotic resistance (AMR) is a global health threat and requires urgent measures. The aim of this study was the generation of up-to-date sequence information for selected pathogens and AMR genes to be used in the development of a suite of real-time isothermal amplification (LAMP/LEC-LAMP) assays to be conducted on-site.

Methods: At National Institute of Health, in Portugal (PT), *E. coli* and *K. pneumoniae* isolates resistant to 3rd/4th generation-cephalosporins and/or to carbapenemes and/or to colistin from human and animal origin were sequenced using a MiSeq Illumina platform. Bioinformatic analysis was performed using command line pipelines and the respective results are being shared among partners.

Results: Overall, three PT WorldCOM AMR gene sequence databases were generated and are available for use in the development and performance evaluation of multiplex assays for pathogens and AMR-encoding genes. This nucleotide sequence information comprised isolates from clinical settings, including hospital surfaces. Among the 187 isolates (38 *E. coli* and 149 *K. pneumoniae*), 99 showed the presence of the $bla_{\text{KPC-3}}$ carbapenemase; $bla_{\text{NDM-type}}$, $bla_{\text{OXA-48-type}}$, and $bla_{\text{VIM-type}}$ were also identified. Mostly of the isolates were extended-spectrum-β-lactamase (ESBL) co-producers: $bla_{\text{CTX-M-15}}$, n=77; $bla_{\text{OXA-9}}$, n=69. Results obtained allowed the identification of not only AMR genes, but also plasmids (e.g., IncFIA, IncN2, IncR), virulence factors, and phages.

Conclusions: Well phenotypically and genotypically characterized bacterial pathogens (*E. coli* and *K. pneumoniae*) isolated from various settings will be used in the development of new LAMP/LEC-LAMP-based assays, which will be suitable for use in a laboratory setting and on-site for both humans and animals.

Funding: This project is funded by One Health EJP

[P092] DIVERSITY OF BACTERIAL COMMUNITIES AND GENES ENCODING AMR IN DIFFERENT ENVIRONMENTAL COMPARTMENTS ALONG THE FOOD/FEED CHAIN

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Aim: The aim of this study was to determine the microbial biodiversity and naturally occurring antibiotic resistance (AR)-encoding genes background load along the food/feed chain in an open-air agricultural testing catchment (HOAL).

Methods: Samples were collected in two time points during the year 2020 from various – but interconnected – environmental compartments of the food/feed chain within an HOAL catchment, such as air, pig feces, manure, soil, water, crops, and feed. All samples were homogenized, diluted and plated in selective media. Colonies were selected and identification made by MALDI-TOF. Antibiotic susceptibility (AST) was assessed by disc diffusion and/or MIC methods. DNA was extracted for further molecular and genomic characterization.

Results: A total of 753 Gram-negative strains were isolated. The highest number of bacteria were from families of *Enterobacteriaceae*(44.1%), *Morganellaceae*(15.5%), *Moraxellaceae*(15.5%), and *Pseudomonadaceae*(9.3%). *Enterobacteriaceae* and *Pseudomonadaceae* were present in all but one of the tested compartments (ground water and feed, respectively). Of notice, 43.1% of *Enterobacteriaceae* identified were isolated from a selective medium supplemented with colistin 0.5mg/L and 8.7% were selected with cefotaxime 2mg/L. Extended-spectrum β-lactamase (ESBL)-producing strains were identified. Regarding Gram-positive bacteria, we identified mainly *Enterococcaceae* (46.7%), *Bacillaceae* (30.6%), and *Streptococcaceae* (13.9%). Pig faces and manure were the compartments with higher bacterial biodiversity. Phenotypic AST revealed the presence of resistances against several AR classes. Based on this data, strains were classified as multidrug-resistant.

Conclusions: The investigation of clinically important AR-encoding genes by PCR-amplification and whole-genome-sequencing of selected strains will contribute to clarify the resistome and microbial biodiversity in the tested environmental compartments.

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[P093] ACQUIRED 16S RRNA METHYLTRANSFERASE ARMA MAINTAINED FOR A DECADE IN A VETERINARY HOSPITAL VIA AN INCR PLASMID

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Aim: Horizontal gene transfer through plasmids plays a pivotal role in antimicrobial resistance (AMR). The hospital environment is a major reservoir for plasmid-mediated antibiotic resistance. IncR plasmids carrying different AMR genes within the *Enterobacteriaceae* family have been reported worldwide. The aim of this study was to perform a comparative analysis of IncR plasmids to understand their epidemiological dynamics.

Methods: Five high-level aminoglycoside resistant *Klebsiella pneumoniae* were obtained from cats and dogs between 2008 and 2010. Additionally, a high-level aminoglycoside resistant *Enterobacter cloacae* from a horse was identified in 2018 at the same hospital. Isolates were sequenced using Illumina and Nanopore technologies to resolve genomic structures and to identify plasmids. Chromosomes and plasmids were annotated using Prokka and then compared with Easyfig and BRIG.

Results: *K. pneumoniae* isolates carried *armA* integrated in IncR plasmids, which shared an identical backbone. All plasmids presented a multi-resistance region containing bla_{DHA-1} **β-lactamase gene and** *qnrB4*, together with *armA*, always flanked by two direct repeats of IS26. This region was found in some other plasmids of *K. pneumoniae* isolates worldwide, mainly in Asia. Interestingly, the *E. cloacae* complex isolate harboured an IncR plasmid with high similarity to those found 10 years earlier in cats and dogs at the same veterinary hospital.

Conclusions: We report evidence of the horizontal transfer of an IncR plasmid between different bacterial species of the *Enterobacteriaceae* family in a clinical environment, where this genetic platform was responsible for the maintenance and spread of *armA* in a single veterinary hospital for a decade.

[P094] RING TRIAL TO VALIDATE THE DISK DIFFUSION METHOD FOR CLOSTRIDIOIDES DIFFICILE

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Aim: A disk diffusion method (DD) for the antimicrobial susceptibility testing of *Clostridioides (C.) difficile* as cost-effective alternative to the current gold standard, agar dilution, was established within the OHEJP IMPART project. Here, we conducted an international ring trial to evaluate and validate this method in terms of applicability, robustness and interlaboratory reproducibility.

Methods: Seven expert laboratories from Germany, Denmark and Portugal participated in the ring trial and determined inhibition zone diameters (IZDs) of eight *C. difficile* strains for eight different antimicrobials (clindamycin, erythromycin, imipenem, moxifloxacin, metronidazole, rifampicin, tetracycline, and vancomycin) using a provided DD protocol.

Results: All participants were able to correctly apply the DD protocol and analyse the eight *C. difficile* test strains for all given antimicrobials except one strain/antimicrobial combination in one lab. Resulting IZD standard deviations (SD) were below 5 mm across all participants and antimicrobials. Metronidazole shows the highest SD independent of the investigated strain. The choice of the equipment to generate anaerobic conditions had a significant impact to the measured IZDs and SDs within the ring trial.

Conclusions: The DD protocol was successfully applied by seven independend laboratories to determine the IZDs of eight *C. difficile* strains with different resistance profiles against eight antimicrobials of different antimicrobial classes. We found overall low SDs and a high reproducibility in dependence of the antimicrobial (e.g., Metronidazole) as well as the equipment used to generate anaerobic conditions.

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Funding: This project is funded by the One Health EJP, IMPART project

[P095] REEMERGING BOVINE BRUCELLOSIS AND CONCOMITANT ONSET OF COVID-19 PANDEMIC SUGGEST INCLUDING ECONOMIC HEALTH IN THE ONE HEALTH APPROACH

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Aim: One health overlaps the EC green deal plan and its relaunched farm to fork strategy aimed at engaging consumers in the choice of local, healthy and sustainable products. However, this model is unlikely to be adopted in areas where brucellosis persists and, consequently, the prices of dairy products are not competitive due to the decrease of breeding yield. Authors suggest the inclusion of "economic health" in the one health approach, in order to fully evaluate and promote specific eradication programmes in areas at high prevalence of brucellosis, but economically unable to sustain the eradication costs. In this way, the alignment of EU policies can be achieved

Methods: Official data concerning bovine brucellosis in EU, Italy and Molise and concerning feed prices have been acquired

Results: Brucellosis in cattle is still endemic in few ms. In 2018, molise had only 1 positive head in the province of isernia, while the province of campobasso was officially brucellosis free (obf) in cattle. 36 outbreaks were registered in the following 18 months; stamping out was carried out in 16 livestock. A critical economic situation followed, due to the concomitant onset of covid-19 pandemic, during which farmers experienced losses and the increase of feed price

Conclusions: In non-obf ms, endemic brucellosis in animals and people has been aggravated by the concomitant covid-19 pandemic: By including the economic health in the one health approach, appropriate initiatives can be promoted to achieve eradication and the consequent alignment of EU policies (one health, EC green deal, farm to fork strategy)

[P096] BIOPIGEE: MODELLING OF THE COST AND EFFECTIVENESS OF BIOSECURITY MEASURES

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Aim: One aim of the European Joint Programme (EJP) funded project BIOPIGEE, is to assess the cost-effectiveness of standard and specific biosecurity measures, in relation to the prevalence reduction of *Salmonella* and Hepatitis E virus (HEV) along the pig supply chain. To fulfill this aim, we propose a modelling framework that brings together currently available models developed by different European institutes and adapts and integrate them for this purpose.

Methods: The framework brings together three models; a) a between herd network model (SimInf), b) a farm-to-consumption quantitative microbial risk assessment (QMRA) and c) an economic model. The SimInf and QMRA models simulate transmission and spread along the food chain and the effectiveness of the biosecurity measures, using data collected elsewhere in the BIOPIGEE project. The model output regarding the reduction in risk of human exposure to products from infected animals due to improvement of biosecurity protocols, will be evaluated, along with their costs and benefits using the economic model. The final output will be a comparison of the cost-effectiveness of multiple intervention measures.

Results: While the framework has been developed, significant data gaps and/or data sharing issues have been identified, such as the costs and quantification of the monetary benefit of biosecurity measures and detailed pig movement data.

Conclusions: More epidemiological and economic data are needed to address these information gap or alternative modelling methodologies must be implemented. The latter, will come at the cost of increasing the uncertainty about the model results, leading to less robust conclusions for policy makers and veterinarians.

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[P097] AN INVESTIGATION INTO THE PREVALENCE, VIRULENCE CHARACTERISTICS AND SHEDDING DYNAMICS OF SHIGA-TOXIN PRODUCING ESCHERICHIA COLI AND THE SUPER-SHEDDING OF SEROGROUPS O157 AND O26 IN IRISH SHEEP

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Aim: Shiga-toxin producing *Escherichia coli* (STEC) are a diverse group of pathogenic bacteria. Serogroups O157 and O26 have attracted particular interest due to their prevalence in human disease. Sheep harbour the organism in the recto-anal junction (RAJ) and shed variable quantities of the pathogen *via* faecal excretion. Some animals, known as 'super-shedders', may contribute higher quantities (>Log_{10}4 colony forming units g^{-1} faeces) of STEC and represent an increased transmission risk. This study assessed the shedding of STEC and super-shedding of serogroups O157 and O26 in Irish sheep and the risk factors underpinning shedding dynamics.

Methods: RAJ samples were collected over 24 months from two ovine slaughtering facilities. Metadata for each animal was recorded. Samples were enriched in modified Tryptone Soya Broth with novobiocin (16µg/ml) at 41.5°C for 5 hours and subjected to a quantitative RT-PCR assay to enumerate *E. coli* O157 and O26. Enrichment continued for 24 hours and samples were assessed for *stx1*, *stx2* and *eaeA via* qRT-PCR followed by cultural isolation of positive colonies.

Results: In total, 704/840 animals were qRT-PCR positive for STEC and 363/840 animals were subsequently culture positive. Some 438 STEC colonies of varying seropathotypes were identified. Five animals harboured STEC O157 and three were super-shedders. *Post-hoc* statistical analysis observed that younger animals are more likely to be STEC positive and that STEC carriage is most prevalent during the summer months.

Conclusions: This research indicates that a high prevalence of STEC is circulating in Irish sheep and further investigation of their zoonotic potential is necessary.

[P098] CHARACTERISATION OF SHIGA-TOXIN PRODUCING ESCHERICHIA COLI (STEC) ISOLATED FROM IRISH SHEEP USING A WHOLE-GENOME SEQUENCE-BASED APPROACH

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Aim: Shiga-toxin producing *Escherichia coli* (STEC) are a significant zoonotic pathogen. Disease causing strains typically harbour a combination of virulence factors including Shiga toxins (Stx), the intimin protein, a type 3 secretion system (T3SS) and a combination of non-LEE encoded (Nle) proteins. A high STEC prevalence rate has previously been observed in Irish sheep. However, limited information is available on the predominant STEC serogroups harboured by this ruminant host and the zoonotic potential of the colonising strains. To address this, a cohort of STEC isolates collected from Irish sheep were analysed using whole-genome sequencing (WGS).

Methods: STEC isolates (N=199) were sequenced on the Illumina MiSeq platform. Raw reads were assessed using FastQC (v0.11.9) and trimmed using Fastp (v0.20.1). Trimmed, paired reads were assembled using SPAdes (v3.14.1) and quality assessed by QUAST (v5.0.2). Annotation was performed using the ABRIcate tool (v1.0.1). Sequence type was determined using the mlst (v2.19.0) tool and cgMLST was performed using Roary (v3.13.0).

Results: In total, 177/199 genomes were confirmed as STEC following sequencing. Thirty-two different O-serogroups and seventeen H-antigens were identified, with a total of thirty-seven different serotypes reported. Serotype O91:H14 was the most frequently observed. Six shiga-toxin gene variants were reported and variant $stx_{1C\text{-}OX3:H8}$ was the most prevalent. The *ecp* operon was ubiquitously distributed among strains. Genes involved in hemolysis/iron uptake (*hlyA, fepC, fyuA, irp2*), adherence/agglutination (*ompT, agn43, cah*) and enterotoxins (*senB, espL2*) were also widely distributed.

Conclusions: These results highlight the diverse range of STEC colonising Irish sheep and the zoonotic potential of the circulating strains.

[P099] HEPATITIS E VIRUS INFECTION DYNAMICS IN INDIVIDUAL PIGS AND PIG FARMS

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Aim: To review current knowledge on hepatitis E virus (HEV) infections in pigs and infection dynamics in pig farms and discuss the implications of this knowledge for future on farm risk mitigation of HEV.

Methods: A literature search was done using PubMed, Scopus and CabAbstracts. Search terms were 'Hepatitis E virus', 'pigs', 'herd', 'transmission', 'infection dynamics', and their synonyms. Moreover, the references of key articles were used to retrieve additional sources. All available English scientific literature published until May 2020 that was considered relevant was included.

Results: Pigs are orally infected and start shedding HEV after a latent period of 1 - 2 weeks. Shedding occurs via feces and urine and lasts on average 1 – 3 weeks with a maximum up to 7 weeks. Seroconversion starts ~3 weeks past exposure. In farms, maternal immunity and a low proportion of HEV shedding sows prevent most infections of sucklers and young weaners. The maximum estimated prevalence is predicted in pigs of 11 weeks old (young fatteners). However, infection dynamics differ notably between farms and depend on exposure to contaminated environments, possibilities of contacts between farm compartments and (the lack of) other internal biosecurity measures.

Conclusions: To prevent transmission of HEV between pigs and reduce the risk of human exposure to HEV, risk mitigation should focus on improving internal biosecurity. For instance, by better cleaning and disinfection routines and limiting animal mixing, especially during the movement of pigs from the weaning to fattening phase.

[P100] INFLUENCE OF ORAL ADMINISTRATION OF LACTOBACILLUS REUTERI PROBIOTIC STRAINS AND GOS PREBIOTIC ON THE PRESENCE OF SALMONELLA SPP. IN FATENNING PIGS

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Aim: The aim of this study was to investigate the effect of the addition of probiotics *Lactobacillus reuteri* SAP 3319 and *L. reuteri* SAP 3320, alone or in combination with galacto-oligosaccharides (GOS - Clasado) prebiotic on the growth rates of commercial pigs and the presence of pathogenic intestinal bacteria.

Methods: The studies were performed using 4 experimental groups, each consisting of 25 weaned pigs. The study spanned both the growing and fattening periods. Broth cultures of probiotics *Lactobacillus reuteri* SAP 3319 *L. reuteri* SAP 3320 and GOS as a prebiotic were tested. Each pig received at least $1x10^9$ CFU / day of the probiotics via the water system. The number of lactic acid organisms in the faeces were enumerated using De Man, Rogosa and Sharpe agar, the presence of *Salmonella* according to the ISO 6579-1: 2017 methodology.

Results: Indicators of production efficacy, such as food conversion ratio and the incidence of intestinal disorders, were monitored. The numbers of *Lactobacillus* Spp. in the pigs in group 3 (both probiotics plus prebiotic) was the most stable and was found to be above 7.4 CFU / g. At post slaughter no *Salmonella* was detected in the groups treated with the *L. reuteri* SAP 3319 probiotic or the pigs treated with both probiotics and the prebiotic.

Conclusions: Treatment of pigs with *Lactobacillus reuteri* SAP 3319 or a combination of both probiotics and a prebiotic resulted in a reduced incidence of intestinal disorders and the absence of *Salmonella* in the faeces.

[P101] PIG FARM CLASSIFICATION IN ITALY: AN HEV PERSPECTIVE

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Aim: Hepatitis E virus (HEV) is an emerging pathogen in industrialised countries. Humans become infected thorough contaminated food. Pigs and wild-boars are the main reservoirs. In Italy, the geographical distribution of HE is heterogeneous with central regions and Sardinia showing the highest level of occurrence. In our study we investigate if demographic factors of the pig population may explain the observed pattern of HE in humans.

Methods: Official data were obtained from the National Animal Registry (31/12/2020). Pig farms were categorised based on a series of factors including the distribution and prevalence of animals at the different age and production stages, the size, the type of farming activity and their combinations. Pig populations at regional level were characterised and compared based on the distribution of the different farm classes to unravel peculiar demographic structure.

Results: We identified almost 20 different classes of farm with a heterogeneous distribution across the country. The majority of pigs are farmed in large single type units in northern regions, with more than 50% of the farms belonging to few classes. In central and southern regions a more scattered distribution of farm classes was observed, with small to medium population size.

Conclusions: The heterogeneity of pig population among Italian regions reflects important differences in the farming and husbandry practice, which may also influence the transmission dynamic of HEV within and between farms. Further joint integrative analyses based on these findings are ongoing to understand how pig demography may explain the epidemiological pattern of HE in humans.

[P102] UNDERSTANDING AMR FROM THE PERSPECTIVE OF POLICY MAKERS AND STAKEHOLDERS: A MULTINATIONAL APPROACH TO DETERMINE GLOBAL AWARENESS

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Aim: Minimizing the effect of antimicrobial resistance (AMR) requires an adequate policy response that relies on good governance and coordination. We have previously demonstrated a knowledge gap on infectious diseases within the general public, but equivalent data from a policy context are still lacking. The aim of this study is to investigate the role of policy makers and stakeholders in tackling AMR on a global level

Methods: A digital survey was designed to capture the awareness, knowledge, attitude and practices (AKAP) towards AMR among politicians, political advisors and relevant stakeholders. Survey responses were collected between November 2020 and March 2021.

Results: 344 individuals participated in this study with the majority of representatives from the Netherlands, Spain and Myanmar. Overall, participants had sufficient knowledge regarding AMR and reported the importance of political willingness in tackling AMR. More than half (65%) of participants from developing nations reported antibiotics misuse, and almost half (48%) claimed to be unaware of this health problem. All participants emphasized the role of the veterinary sector in particular, as well as the environmental dissemination of antibiotics (residues). The lack of funding resources was especially reported by participants from developing nations.

Conclusions: Inter-regional differences in AKAP regarding AMR exist among politicians, policy advisors and relevant stakeholders. Overall, participants demonstrated to have a sufficient level of knowledge and awareness of AMR. This study characterizes a multi-national policymaker and stakeholder mapping that can be used to propose further policy implementation on various governance levels.

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[P103] FARMED: LONG-READ METAGENOMIC SEQUENCING WORKFLOW FOR THE IDENTIFICATION OF PATHOGENS/AMR ON-SITE

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Aim: Antimicrobial resistance (AMR) is a global health concern with negative consequences to both human and animal health, resulting in economic losses associated with increased healthcare expenses and reduced productivity. Current AMR detection is primarily reliant on time-consuming classical culturing techniques; the development of new tools for real-time detection of resistant pathogens is an EJP priority topic. Third-generation sequencing techniques have the potential to offer real-time detection of pathogens, however their applicability for on-site work is hindered by lack of suitable protocols/equipment for sample processing. FARMED (Fast AMR and Mobile-Element Detection using metagenomics for animal and human on-site tests) aims to develop on-site long-read metagenomics workflows to detect the presence of pathogens/AMR and profile the microbiome of sample matrices.

Methods: We present the development and benchmarking of a rapid on-site DNA extraction workflow for sequencing using the Oxford Nanopore Technologies MinION system for the detection of AMR/pathogens in metagenomic samples. Existing methods were trialled to determine the feasibility of long-read sequencing to detect spiked bacteria in sample matrices.

Results: Preliminary analysis using spiked matrices indicated the suitability of long-read metagenomics methods to detect spiked AMR/pathogens, as well as profile the microbial community of matrices. We review the current literature and commercially available methods for on-site DNA isolation, and use this to develop 'portable' DNA extraction methods, undertaken outside the laboratory.

Conclusions: The workflow developed for bacterial species detection on-site (e.g. human clinics, animal farms, environment) will enable faster and better informed treatment strategies against AMR pathogens.

[P104] PREVALENCE OF TRANSFERABLE MECHANISM OF QUINOLONE RESISTANCE IN ESCHERICHIA COLI ISOLATES COMING FROM GULLS NESTING IN THE CZECH REPUBLIC IN 2018 AND 2019 YEAR-SEPARATED SAMPLINGS

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Aim: Gulls as synanthropic birds represents a valuable indicator of the pollution by antimicrobial resistant bacteria in wildlife. In this study we evaluated prevalence of *Escherichia coli* with transferable mechanism of quinolone resistance (TMQR) in gulls nesting in a colony near to the Nove Mlyny water reservoirs in 2018 and 2019.

Methods: Cloacal samples were taken from gulls in July 2018 (n=72) and May 2019 (n=45). The samples were cultivated on MacConkey agar with ciprofloxacin (0.05 mg/L) and one isolate per plate was recovered and species identified using MALDI-TOF. Representative *E. coli* isolates were sequenced using Illumina platform. SNPs-based phylogeny was performed and presence of antibiotic resistance genes was evaluated.

Results: A total of 95 *E. coli* isolates, 58 from 2018 and 37 from 2019, were obtained. In 2018, TMQR genes were detected in 33% (n=58) of isolates represented by *qnrB19* (21%) and *qnrS1* (12%). In 2019, TMQR occurrence was 41% (n=37) and most prevalent was *qnrS1* (30%) followed by *qnrB19* (11%). Several international zoonotic *E. coli* sequence types (ST) were detected including ST10, ST648, ST117, ST457, ST744, ST58, ST93, ST88, ST1158 and ST155. While some STs were detected in both years, the closest isolates from 2018 and 2019 differed in hundreds of SNPs.

Conclusions: The prevalence of TMQR genes in *E. coli* among gulls in the Czech Republic seems to have an increasing tendency. Gene *qnrB19* was most dominant in 2018 while *qnrS1* was the most common in 2019. Of the concern is the detection of successful *E. coli* STs in the gulls.

[P105] IDENTIFICATION OF THE BEST BIOSECURITY PRACTICES IN SLAUGHTERHOUSE IN REGARDS TO SALMONELLA AND HEPATITIS E VIRUS

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Aim: To collate relevant evidence on the impact of slaughterhouse biosecurity practices on carcass contamination/cross-contamination with *Salmonella* and hepatitis E virus.

Methods: A literature review will be completed to inform a short, focused questionnaire which will be used to collect data from at least seven project partner countries (UK, IT, EE, AT, NL, DE, CZ) on currently implemented pig slaughterhouse biosecurity practices. Results from this questionnaire will be used to revise and formulate a final version, which will be tested at slaughterhouse sampling visits (n = 3) to validate the importance of these measures. Finally, a guidance document and assessment protocol for slaughterhouse biosecurity best practice will be produced, which will be supplied to slaughterhouse industry bodies, regulatory authorities and other partners who might benefit from it.

Results: In total, 67 sources of information were used to form a preliminary literature overview with 209 pieces of relevant information. In the first version of questionnaire, there were 39 questions, which will be reduced to 20-30 questions. First results from the slaughterhouses are expected in the end of April 2021.

Conclusions: Slaughterhouses and regulatory bodies at the moment do not seem to have access to a *Salmonella* and hepatitis E virus oriented check-list, which describes the best biosecurity practices and is based on the latest scientific research.

[P106] THE FOS OPERON MEDIATING UTILIZATION OF SHORT-CHAIN FRUCTOOLIGOSACCHARIDES LOCATED ON BOTH CHROMOSOME AND PLASMID OF MULTI-DRUG RESISTANT BACTERIA

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Aim: The plasmid-mediated metabolism of the short-chain fructooligosaccharides, commonly supplemented to human and animal nutrition as prebiotics, could play a key role in the adaptation of plasmids among multi-drug resistant (MDR) bacteria and promote the spread of antibiotic resistance. The study focused on the occurrence and structure of the *fos* operon among MDR bacteria from various sources.

Methods: The PCR screening was performed to detect the *fosT* gene as part of the *fos* operon in more than 11000 MDR isolates (mostly *Escherichia coli*) obtained between 2005 and 2019 from humans, food-producing and companion animals as well as wildlife and environment in Europe, North and South America, Africa and Australia. The *fosT*-positive isolates were subjected to whole-genome sequencing (WGS) to determine the location of the *fos* operon on plasmids or chromosome.

Results: The *fosT* gene was detected in 304 isolates, namely 165 wildlife, 25 companion, 18 food-producing and 11 zoo animal together with 60 environmental and 25 human isolates. From the total of 188 isolates subjected to WGS, 126 carried a complete *fos* operon consisting of seven *fos* genes. The *fos* operon was both plasmid- and chromosomally-encoded, with the chromosomal variant predominating. The most common extrachromosomal location was detected on IncHI1 and IncF plasmids with combinations of multiple antibiotic resistance genes (ARG). Long-read sequencing will be performed to obtain a complete genetic environment of the *fos* operon.

Conclusions: We demonstrate wide occurrence of the *fos* operon among MDR bacteria from diverse sources and suggest possible co-selection of ARG together with the *fos* operon on plasmids.

[P107] BUILDING POLITICAL WILL FOR ONE HEALTH RISK ANALYSIS SYSTEM

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Aim: Committed support from the government is essential for the implementation of a One Health Risk Analysis System (OHRAS). The lack of political will has been identified as one of the challenges that professionals working in the area of One Health and zoonoses encounter in order to implement a OHRAS. Therefore, in this research we aim to understand how these professionals can build political will for its implementation. The results of this research will be part of a political will tool as an element of the implementation guidelines created by COHESIVE.

Methods: A transdisciplinary research method was applied. Workshops, focus groups and semi-structured interviews (SSIs) were performed in the period of 2019 and 2020. Professionals working in the field of One Health and zoonoses in the European region participated in the different sessions and SSIs. During the sessions, methods such as brainwriting and group discussions were used. The sessions and interviews were recorded and transcribed for thematic analyses.

Results: The preliminary outcomes of the research resulted in practical advices aimed at building political will, such as agenda-setting, improving communication with policy makers, and understanding governments' prioritisation including (financial) resource allocation.

Conclusions: Understanding political will in the context of OHRAS is an essential element for the implementation of OHRAS. Following the proposed guidelines, professionals working in the area of One Health and zoonoses will be able to build political will to support the implementation of OHRAS.

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References:
OHRAS: See ASM 2020 'COHESIVE: Understanding the needs for European implementation guidelines for a One Health Risk Analysis System for zoonoses' https://onehealthejp.eu/wp-content/uploads/2018/12/D3.12-OHEJP-ASM-2020-Abstract-book.pdf
COHESIVE webpage: https://onehealthejp.eu/jip-cohesive/

[P108] BIOFILM FORMING CAPABILITY AND DISINFECTANT TOLERANCE OF PERSISTENT SALMONELLA 13:23:I:- ISOLATES RECOVERED FROM A POULTRY HATCHERY

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Aim: *Salmonella* 13:23:i:- is one of the top five serovars isolated from hatcheries and broiler chicken flocks in Great Britain. The hatchery in this study had difficulty in eradicating resident *S.* 13:23:i:- contamination despite adjustments to their cleaning and disinfection (C&D) regime. Some isolates showed low-level resistance to ciprofloxacin, a critically important antibiotic for the treatment of *Salmonella* infection in people. The study aim was to assess biofilm forming capability and disinfectant tolerance of *S*. 13:23:i:- isolates collected from the environment within this hatchery.

Methods: Twenty *Salmonella* 13:23:i:- isolates, collected over four years from a single hatchery in the United Kingdom, were assessed for biofilm forming capability at 20±1°C and 25±1°C using the crystal violet microplate assay. Two disinfectants, a benzalkonium chloride-based Quaternary Ammonium Compound (QAC) product and a QAC/Glutaraldehyde-combination product, were tested at in-use and recommended concentrations. Disinfectant efficacy was assessed using a tetrazolium chloride microplate biofilm assay and a coupon-based biofilm model using materials found in the hatchery environment.

Results: All isolates demonstrated biofilm forming capability, with the majority classed as good biofilm producers; two isolates were poor biofilm producers. As expected, all isolates demonstrated some tolerance to both disinfectant products, with good biofilm producers showing least susceptibility.

Conclusions: Persistence of *S.* 13:23:i- isolates in the hatchery environment may be facilitated by their biofilm forming capability as biofilms protect bacteria against environmental stresses including disinfectants. Data collected regarding the effectiveness of different disinfectant products, at different concentrations and on different materials, will help define more effective C&D regimes.

[P109] ASSESSMENT OF THE BIOFILM FORMING CAPABILITY OF SALMONELLA ISOLATES SOURCED FROM PIG FARMS IN THE UNITED KINGDOM

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Aim: *Salmonella* can reside as a biofilm in the farm environment, posing a risk of infection to pigs and humans through the food chain. The study aim was to evaluate the biofilm forming capability of wild type isolates of *Salmonella* serovars relevant for pig production.

Methods: Fifteen *Salmonella* Derby and 35 *Salmonella* Typhimurium isolates (ST), including monophasic variants (mST), isolated from pig faecal samples collected on commercial indoor pig farms between 2007 and 2018 were tested. Biofilm forming capability was assessed using the crystal violet microplate assay after incubation for 48 hours at 20±1°C. Biofilms were classified as strong, moderate, weak or not formed. Colony morphology was assessed on Luria-Bertani agar plates supplemented with Congo Red and Coomassie brilliant blue, after 96 hours incubation at 20±1°C. Experiments were repeated twice.

Results: Nine (60%) of the 15 *S.* Derby isolates produced biofilms. Two isolates (13.3%) were classified as moderate biofilm producers and both expressed the RDAR (red, dry and rough) morphotype. All 35 ST/mST isolates produced biofilms. Twenty-nine isolates were classified as moderate biofilm producers and two as strong producers. Of these 23 isolates (65.7%) expressed the RDAR morphotype.

Conclusions: Half of the *Salmonella* isolates tested were good biofilm formers (moderate/strong biofilm producers that expressed RDAR morphotype). Good biofilm forming strains maybe less susceptible to disinfectants than poor biofilm formers, and therefore be more difficult to eliminate from the farm environment. Future work will assess the susceptibility of biofilms from 10 isolates to disinfectant products commonly used on commercial pig farms.

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[P110] DIVERSITY AND DYNAMICS OF CLOSTRIDIOIDES DIFFICILE IN A FARM ENVIRONMENT

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Aim: The recent increase in community-acquired *Clostridioides difficile* infections discloses the shift in this bacterium epidemiology. The commensal nature of this enteric pathogen in most animal and environmental samples reveals the importance of natural occurring reservoirs in the transmission cycle. Our goal was to evaluate the potential of *C. difficile* being a foodborne/zoonotic pathogen and to establish a possible transmission network based on a "one health" approach.

Methods: Samples were obtained from a Portuguese zootechnical station, and collected from different compartments of animal (pigs and others), human and environmental origin. All samples were broth enriched and cultured on selective medium. For each isolate, the toxins profile was evaluated by multiplex-PCR and genetic diversity by PCR-ribotyping and WGS; antibiotic susceptibility was performed.

Results: Ninety-six samples were collected between July-December-2020. The overall *C. difficile* prevalence was 26% (25/96), with all samples harbouring toxigenic strains, except one. Only the human compartment was negative for *C. difficile*. Nine different ribotypes (RT) were found, with some samples (water/soil/cattle) presenting more than one type. RT033 was present in all positive compartments with a prevalence of 84%. Moxifloxacin resistance was detected in two samples, one from a sheep-RT126 and one from a soil sample-RT027.

Conclusions: The presence of RT033 in all compartments associated with the pig barn, suggests a transmission cycle originated in these animals. These findings reveal the potential of *C. difficile* being a zoonotic pathogen. WGS is currently being performed to understand the genetic diversity of this putative zoonotic ribotype and its transmission dynamic.

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[P111] A PRELIMINARY STUDY OF CAMPYLOBACTER SPP. IN DOGS IN PORTUGAL – A ONE HEALTH PERSPECTIVE

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Aim: Campylobacteriosis has long been the most reported zoonotic disease in the European Union. Since poultry is the main reservoir of *Campylobacter*, not much attention has been payed to other sources of infection, like companion animals. Despite these bacteria being mainly considered as commensal in dogs, the development of symptoms like those in humans, is also described. Given the public health and clinical practice concerns, and since no previous epidemiological study in Portugal was reported, we aimed to obtain a first insight of the prevalence and characteristics of this microorganism in different canine populations.

Methods: A total of 125 rectal swabs were collected from dogs hold for companionship and hunting, and from different regions: rural and urban, between September-December 2020. Phenotypic characterization, including antimicrobial susceptibility testing, and genotyping through different molecular techniques, including WGS of a selection of isolates, was performed.

Results: From a total of 32 *Campylobacter* spp. isolates obtained, 14 were identified as *C. jejuni* (44%), of which 93% were resistant to ciprofloxacin, 64% to tetracycline and 57% to ampicillin, with three isolates being multidrug resistant. Comparison of the phenotypic and genotypic traits with human isolates obtained in Portugal during 2020, revealed great similarity between both sources. Particularly relevant results were obtained by wgMLST analysis, which allowed the identification of isolates from human and dogs sharing high genetic proximity.

Conclusions: Despite being only a preliminary study, the close epidemiological relationship between the isolates obtained from both species revealed that dogs could be a more relevant source of *Campylobacter* to human than currently considered.

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[P112] MILK SAMPLES FOR IMMUNOLOGICAL DIAGNOSIS OF CAPRINE TUBERCULOSIS

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Aim: The objective of this study was to evaluate the performance of an experimental antibody (Ab)-based test for TB diagnosis in goats using milk samples.

Methods: individual serum and milk samples from low and high prevalence TB-infected caprine herds were assayed using an indirect ELISA that detects antibodies against a protein complex purified from bovine Purified Protein Derivative (CZV) named P22, (P22 ELISA) and compared with cell-based diagnostic tests. Moreover, bulk milk tank samples were assayed to evaluate them for herd screening. To evaluate the Ab levels in milk during the lactation period and determine whether time of sampling may affect the results, goat milk samples (n=48) from a high TB prevalence dairy herd were collected each 2 weeks during 6 months and assayed using P22 ELISA.

Results: Sensitivity of P22 ELISA was similar using serum [(78.5% (95% CI 64.1-88.3)] and milk [(83.3% (95% CI 69.4-91.7)] samples, suggesting the usefulness of milk as alternative sample for TB diagnosis in dairy herds. Moreover, P22 bulk milk tank samples detected up to 4-6% TB herd prevalence. Finally, Ab levels in milk did not show significant variations along the study.

Conclusions: Results from the study suggested that milk samples are valuable for Ab-based TB diagnosis in caprine dairy herds and that the Ab detection is not affected by the lactation stage. Moreover, bulk milk tank samples could be useful to define the status of the herd. P22 ELISA using milk samples demonstrated to be a valuable TB diagnostic tool in caprine dairy herds.

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[P113] ANTIBODY-BASED DIAGNOSIS OF CAPRINE TUBERCULOSIS USING ORAL FLUID SAMPLES

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Aim: The objective of this study was to evaluate the performance of an experimental antibody (Ab)-based test for tuberculosis (TB) diagnosis in goats using oral fluid samples.

Methods: Individual serum and oral fluid samples from TB-free and TB-infected caprine herds were analysed using an indirect ELISA that detects Ab against a protein complex purified from bovine Purified Protein Derivative (CZV, Spain) named P22, (P22 ELISA) and compared with severity of lesions observed in lungs and head and pulmonary lymph nodes at the *post-mortem* analysis. Both herds were subjected to single and comparative intradermal tuberculin tests and interferon-gamma release assay too.

Results: The sensitivity and specificity were 34.4% (95% CI 22.4-45.6) and 100% (95% CI 97.4-100) respectively. Similar Ab values in oral fluid and serum samples (p=0.852) were obtained in animals from TB-free herd. Animals with higher levels of Ab in oral fluid showed a significantly higher TB lesion score (p=0.018) and TB-infected animals showed higher Ab levels in serum compared to oral fluid samples (p<0.0001). In fact, only taking into account the setting of animals showing severe lesions (n=16), the ELISA showed a Se of 75% (95% CI 53.7-96.2).

Conclusions: Results from the present study suggested that specific Ab detection using P22 ELISA in oral fluid samples has a limited value for TB diagnosis in goats although its performance improves in animals with severe lesions in the lungs.

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[P114] DIFFERENTIAL DETECTION OF CTX-M GROUP 1 VARIANTS FROM ESCHERICHIA COLI ISOLATES USING A MULTIPLEX LOOP-PRIMER ENDONUCLEASE CLEAVAGE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LEC-LAMP) ASSAY

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Aim: Cefotaximases (CTX-Ms) are plasmid-encoded extended-spectrum beta-lactamase (ESBL) enzymes found in Enterobacteriaceae such as *Escherichia coli* that confer resistance to third-generation cephalosporin antibiotics. CTX-M enzymes are classified into five groups; CTX-M-1, 2, 8, 9 and 25. The rapid emergence and dissemination of CTX-M group 1 variants $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$, typically associated with animal and human infection, respectively, is a global public-health concern and highlights the requirement for effective diagnostic tools. However, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ variants are almost identical in nucleotide sequence and difficult to differentiate using conventional molecular diagnostics. Loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) is a recently developed technology that enables multiplex pathogen detection with single-base specificity and portable on-site testing applications. In this study we have developed an internally controlled multiplex LEC-LAMP assay for the differential detection of $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ variants in a single reaction.

Methods: Analytical specificity and sensitivity of the LEC-LAMP assay was established using clinical and environmental *E. coli* isolates from Ireland and Central Germany.

Results: The LEC-LAMP assay demonstrated specific differential detection of both variants at high bacterial load concentrations of 10^6 genome copies, and low-level detection for each variant of 10 genome copies per reaction in approximately 15-20 min.

Conclusions: This is the first report of portable diagnostics technology that can provide effective differential detection of CTX-M group 1 variants $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$. The LEC-LAMP assay will be further validated using $bla_{\text{CTX-M}}$ positive bovine and alpaca faecal samples, and evaluated for on-site agricultural faecal sample testing in combination with portable instrumentation.

Funding: This project is supported by the One Health EJP

[P115] THE OCCURRENCE OF POTENTIAL VIRULENCE GENES IN EMERGING ZOONOTIC PATHOGENS SHEWANELLA SPP. COLLECTED FROM FISH

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Aim: Bacteria *Shewanella* are the opportunistic pathogens of fish. They can also be risk to human health, mainly causing skin and soft tissue infections. Some phenotypic virulence factors of this group of bacteria have been identified. However, the genetic basis of the pathogenicity is still unknown. The aim of our study was to determinate the presence of potential virulence genes in different species of Shewanella.

Methods: A total of 85 isolates collected from marine and freshwater fish, representing the following species were analyzed: *S. baltica*, *S. oneidensis*, *S. xiamenensis, S. glacialipiscicola* and *Shewanella* sp.. Whole genome sequencing by Illumina platform (MiSeq) was conducted. The *in silico* analysis for the presence of virulence genes using the Virulence Factor Database VFDB were performed.

Results: Genome analysis revealed 22 potential virulence genes. The different chromosomal genes related to fimbriae (*pilT*, *tapT*), flagella (*cheW*, *cheY*, *fliG*, *fliM*, *fliN*, *flmH*, *motA*), outer cell membrane (*ddhA*, *ddhB*, *fcl*, *gmd*), protein secretion systems (*exeG, vscN2*, *vscS2*, *hcp-2*, *vipB*), heat shock protein (*htpB*), elongation factor (*tufA*) and the low molecular weight inductor AI-2 (*luxS*) were detected. The presence of the genes was correlated with particular *Shewanella* species.

Conclusions: Our studies confirm occurrence of potential virulence genes in *Shewanella* isolated from fish and indicate on the need for in-depth analysis of pathogenicity mechanisms of this pathogen.

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[P116] RESEARCH BASED UNIVERSITY NETWORK FOR ONE HEALTH

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Aim: To make University of Helsinki one of the most recognized universities in the world regarding one health research by 2025.

Methods: In a competitive research grant call, a proposal was submitted to profile one health research in the University of Helsinki. Five faculties (veterinary, medicine, agriculture, pharmacy and social sciences) out of the 11 faculties of the university were involved in writing the application. The core of the proposal consisted of excellent research groups already active for a long time, especially in the areas of food safety, translational medicine and animal health and welfare. The proposal was therefore based on existing scientific excellence and the greatest potential for break through scientific innovations in one health. Regarding food safety, antimicrobial resistance, risk management in food safety and environmental were profiled and prioritized. Within translational medicine, modelling of spontaneous diseases occurring in natural populations were prioritized, especially in neurological diseases and emerging infectious diseases. Regarding animal health and welfare, animal welfare, ruminant health and medication safety and effectiveness were emphasized.

Results: The proposal was accepted in 2018 – Helsinki One Health was born. Eight new tenure track professors have been appointed in Helsinki One Health by 2020. Societal impact of the appointed young professors has been most visible in their appearance regarding the COVID-19 pandemics in the Finnish and international media. Furthermore, the most important collaborative partners, taking part in the recruitments described, include Food Safety Authority, Natural Resources Institute and Finnish Institute for Health and Welfare. Furthermore, we have received significant resources, input and support for the activities by other public and private organizations and companies and started to build international collaborations via the Una Europa network.

Conclusions: Our experience shows that One Health can be a very successful area for profiling research at a university and in national settings. The current strategic steps include internationalization of activities, an investment in advancement of open data policy and paving way for researchers to access health records and biobanks regarding spontaneous diseases in natural populations.

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[P117] SOURCE ATTRIBUTION OF ESBL-PRODUCING ESCHERICHIA COLI IN GERMANY

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Aim: The goal of the present study was to develop a source attribution model to analyse the connection of Extended Spectrum Beta-Lactamases (ESBL) producing *E.coli* bacteria in the general population and in different sources in Germany.

Methods: Pheno- and genotypic characteristics of ESBL-producing *E. coli* from healthy livestock animals (broilers, fattened pigs, dairy cattle and beef cattle), horses and dogs were used. As regards humans, focus was laid on isolates from the healthy community and patients with nosocomial infections. The developed source attribution model calculates the number of human cases that can be attributed to each of the potential sources based on the assumption that there is a pathway from these sources into the general population.

Results: All sources may contribute to the human cases, nevertheless, nosocomial infections, when considered as a source, showed to be more closely linked to the human cases than the animal sources. Still, quite a considerable number of cases could not be explained by any of the sources considered.

Conclusions: The present study showed that a One Health approach is necessary to develop source attribution models further due to the necessity to include both human and animal data as well as environmental sources. We have developed an approach that can support assessing further trends and we can now better understand the individual contributions of livestock, pets and other humans in the transmission of ESBL *E.coli* bacteria into the general population.

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[P118] MULTICENTRE EVALUATION OF CULTURE BASED METHODS TO SELECTIVELY ISOLATE COLISTIN-RESISTANT ENTEROBACTERIACEAE FROM FOOD PRODUCING ANIMALS AND FOOD PRODUCTS

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Aim: The aim of this study was to compare within the OHEJP-IMPART project screening methods to selectively isolate acquired colistin-resistant Enterobacteriaceae from caecal and meat samples, in order to achieve an effective protocol that could be used in routine surveillance.

Methods: A two-step method, that involved a pre-screening multiplex PCR of a selective overnight enrichment followed by isolation on selective agar media, have been compared in a multicentre trial to detect acquired-colistin resistant Enterobacteriaceae spiked in caecal content or meat samples. Species identification was confirmed by MALDI-TOF, biochemical or genotypic tests. Colistin resistance was verified by broth microdilution and multiplex PCR. Performance of the method was evaluated from sensitivity and specificity of the PCR results as well as the sensitivity of the isolation step for expected positive samples.

Results: Twelve laboratories participated to the multicentre evaluation. The specificity of the PCR was 100%. PCR showed better sensitivity on caecal (95%) compared to meat samples (60%). The sensitivity of the selective agar media for the detection of colistin-resistant Enterobacteriaceae varied according to the bacteria-gene combinations and matrices. Overall, CHROMID® Colistin R showed a better sensitivity (86%) to selectively isolate mcr-positive strains than CHROMagarTM COL-APSE (75%) and COLISTIGRAM (70%).

Conclusions: Given the number of samples to be analysed in a monitoring program, a pre-screening PCR gives an added value by saving time and consumables. CHROMID® Colistin R showed the best sensitivity of the tested commercial agar plates for selective isolation of mcr-positive strains from caecal or meat samples. Further trials including a broader range of colistin-resistant strains and a larger panel of matrices would assess the robustness of the method.

Funding: This project is supported by the One Health EJP

[P119] ZOONOTIC SPREAD OF MULTI-RESISTANT CLOSTRIDIOIDIES DIFFICILE

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Aim: To investigate the zoonotic potential of *C. difficile* and its role in horizontal gene transfer (HGT) of antimicrobial resistance genes (ARGs).

Methods: In total 330 fecal samples were collected during 2020 from nine pig farms in Denmark. Isolates were subjected to whole-genome sequencing (Illumina) allowing determination of multilocus sequence type (MLST), toxin profile, ARGs and core genome MLST (cgMLST). cgMLST types were compared to 800 human clinical isolates from same period.

Results: *C. difficile* was isolated in twelve samples (4%). All isolates were toxigenic (*tcdA+, tcdB+*), four were binary toxin (*cdtA/B*) positive. Six different sequence types (STs) were found: ST11 (n=4), ST6 (n=3), ST7, ST13, ST36, ST49 and a novel ST (each n=1). The six known STs were also found in human isolates. The minimum number of cgMLST alleles between pig and human isolate varied from two to 49. Three ST11 isolates differed by only two or three alleles. Nine different ARGs were observed and nine isolates contained at least one ARG. Fluoroquinolone, tetracycline and macrolide ARGs were most common among pig isolates and were also found in human isolates.

Conclusions: ST11 (equivalent to PCR ribotype 078) was the most common porcine type and the third most common type in humans in Denmark and three isolate pairs were within plausible cgMLST-based transmission range. These results support that pigs constitute a zoonotic reservoir for *C. difficile* and suggest that porcine *C. difficile* might play a role in HGT of ARGs to human *C. difficile* isolates.

[P120] DYNAMICS OF QUINOLONE- AND CEPHALOSPORIN-RESISTANT ESCHERICHIA COLI CARRIAGE ALONG THE PRODUCTION CHAIN IN THURINGIAN PIGSTIES

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Aim: In 2018, the German government restricted veterinary usage of fluoroquinolones (FQ) and certain cephalosporins (CS) to fight rising prevalences of antimicrobial resistance (AMR). Thereafter, tonnages of these AMs delivered to veterinarians significantly declined. AMR determinants (RD) can, however, be retained in the absence of the respective antimicrobial (AM) through co-selection or cross-resistance. With this in mind, we studied the dynamics and persistence of FQ- and CS-resistant *Escherichia coli* strains during fattening runs in Thuringian pigsties.

Methods: Pooled faecal samples from three consecutive fattening runs at one conventional (C2) and two organic (B1; B2) farms were collected over a period of 16 months and screened for indicator *E. coli* on Gassner plates containing enrofloxacin, ceftiofur or cefquinome. After determining the percentage of FQ- and CS-resistant bacteria by colony counting, the resistance profiles of single strains were phenotypically assessed using a commercial diagnostic system for antibiotic susceptibility testing (AST) with 16 AMs (13 classes according to WHO categorization) important in veterinary medicine. Additional genotypic comparison via MLVA-PCR and plasmid profiling allowed the selection of strains for whole genome sequencing (WGS).

Results: On all farms, the prevalences of FQ-/CS-resistant bacteria were generally higher in piglets and declined thereafter. A total of 301 FQ- and/or CS-resistant strains were isolated from all pigsties (n=142/111/48 from C2/B1/B2, respectively). Despite the selection for resistant strains, a large phylogenetic heterogeneity, reflected by 60 different MLVA-patterns (each with more than 98% similarity and containing 1 - 55 strains), was observed. FQ resistance was mutational (*gyrA*, *parC*, *parE*) or plasmid-encoded (*qnrS*, *aac(6')-Ib-cr*), while CS resistance resulted from bla_{CTX-M} (70% $bla_{CTX-M-1}$, 30% $bla_{CTX-M-15}$ – detected via Loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC LAMP)). Phenotypic AST, as well as WGS analysis, revealed the presence of additional resistances against several AM classes. Based on this data, all strains classified as multidrug-resistant. Notably, less than 1% of the strains were resistant to colistin, tulathromycin or amoxicillin-clavulanic acid.

Conclusions: Even after the government amendment, CS- and FQ-resistant strains remain numerous in commensal bacteria of Thuringian pigs. With this in mind, further action fighting AMR presence should consider not just AM usage, but also the respective AMR mechanisms as well as their transmission dynamics.

[P121] PREVALENCE AND CHARACTERIZATION OF PATHOGENIC ESCHERICHIA COLI AND SALMONELLA SPP. IN WILD ANIMALS IN MAINLAND PORTUGAL

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Aim: To evaluate the prevalence and characterize pathogenic *Escherichia coli* and *Salmonella spp.* in different animal-wildlife species in mainland Portugal.

Methods: Between June 2020-January 2021, 205 faecal samples were collected from wild boars (N=39), deer (N=62), and wild birds (N=104). Isolation was performed in selective and non-selective medium, followed by identification of virulence genes of *E.coli* by multiplex PCR, and *Salmonella* serotyping. Antimicrobial susceptibility of *E. coli*, and *Salmonella* isolates was performed according to EUCAST recommendations. PCR for the detection of *mcr* genes was also performed. Whole-genome sequencing is in progress to investigate the presence of AMR genes, multilocus sequence type and phylogeny of the isolates.

Results: The overall occurrence rate of *E.coli* and *Salmonella* was 26.8% (55/205) and 2.9% (6/205), respectively. In wild boars, 7.7% EPEC, 5.1% STEC, 5.1% ETEC, and 5.1% *Salmonella* (*S*.Schleissheim and *S*.Enteritidis) where detected, while in deer the rates were 53.2% STEC, 11.3% EPEC, and 1.6% *Salmonella* (*S*.Schleissheim). In wild birds, STEC were identified in 3.8% of the isolates, EPEC in 1.0%, EAEC in 1.0%, and *Salmonella* in 2.9% (*S*.Typhimurium, *S*.Litchfield, and *S*.IIIb61:k:?). Preliminary results for AMR testing showed that none *Salmonella* isolates were MDR or harbours *mcr* genes. AMR testing for *E.coli* is is ongoing.

Conclusions: Wild animals can be regarded as sentinel species and environmental health indicators, representing a potential pathogens reservoirs for domestic tic animal and human pathogens. As they are increasingly closer to residential areas, it is important to monitor the presence of theses common zoonotic pathogens following a One Health approach.

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[P122] NATURAL HABITATS FOR DETECTION OF EMERGING BRUCELLA SPECIES: A NEW STRATEGY TO IDENTIFY PUTATIVE THREATS (IDEMBRU)

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Aim: The IDEMBRU project focusses on new brucellosis threats, aiming to develop a toolkit to detect and characterize emerging *Brucella* species and reservoirs. These threats comprise *Brucella* atypical and re-emerging classical species associated with atypical animal hosts or humans and new consumption habits.

Methods: Considering bibliographic data and latest clinical case reports, the sampling strategy was defined. Target habitats were selected considering a "One Health" approach in order to cover animal and environmental sources. A common database for all samples was created.

Results: Three habitats were defined in order to screen i) forest, ii) fresh water biotops and iii) coastal regions. From nnvironment soil and water will be collected, while animal samples include swabs, tissues and faeces from various species. Forest habitat samples include wild forest canids, suids and ruminants, rodents and lagomorphs. These potential reservoirs are complemented by soil samples to detect environmental *Brucella*-like bacteria. Fresh water habitats sampling includes rodents and amphibian samples together with water and soil. Filter feeders, sea turtles and marine mammals are considered in coastal regions. An epidemiological questionnaire and associated SOPs for sample collection and identification were prepared. The collection of new samples started in Bulgaria, France, Germany, Italy, and Portugal. Moreover, the screening strategy was completed by existing collections of DNA, tissues and strains.

Conclusions: Following definition of the three primary habitats, IDEMBRU partners launched the collection of samples. At the next step, an exhaustive *Brucella* screening and characterisation will be performed to define the host/environmental sources for developement of the toolkit.

[P123] EVALUATION OF THE PERFORMANCE OF SLAUGHTERHOUSE SURVEILLANCE FOR BOVINE TUBERCULOSIS DETECTION IN CASTILLA Y LEON, SPAIN.

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Aim: Abattoir post-mortem inspection (PMI) plays a pivotal role in maintaining food safety but also animal health, by ensuring animal products are produced according to certain standards. However, ensuring adequate PMI performance is challenging due to the multiple factors that can affect it. Here, we assessed the performance of PMI in the frame of the bovine tuberculosis (bTB) control program to determine between-abattoir variability and associated factors through the assessment of relative efficiencies in the detection of bTB-compatible lesions and subsequent laboratory confirmation in Castilla y Leon, Spain, during 2010-2017.

Methods: The slaughtered population was split based on ante-mortem tests results, and two generalized linear multivariable mixed models were fitted to reactor and non-reactor subpopulations to calculate the abattoir-specific risk of lesion detection and laboratory confirmation while accounting for the effect of confounding variables.

Results: Throughout the 8-year period, ~30,000 reactors and >2.8 million non-reactors were culled. Bovine TB-like lesions were detected in 4,710 (16%) reactors and 828 (0.03%) non-reactor animals, and >95% were confirmed through bacteriology. Detection risk was associated with animal subpopulation, animal breed and age, farm type and size, and year and season of slaughter. Still, PMI varied mostly depending on the abattoir, with probabilities of detection ranging from 603 to 3,070 per 10,000 animals (reactors) and 0.2-16.1 per 10,000 animals (non-reactors).

Conclusions: Results obtained here demonstrate between-abattoir PMI performance for bTB detection, a food safety and animal health important issue, even after accounting for animal-associated characteristics, demonstrating the need for continued efforts to ensure PMI performance.

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[P124] HISTOMORPHOLOGICAL CHANGES IN THE BURSA OF FABRIZIO ASSOCIATED WITH SALMONELLA TYPHIMURIUM INFECTION IN ANIMALS FEED WITH A NUTRACEUTICAL DERIVED FROM THE OLIVE OIL PRODUCTION

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Aim: *Salmonella* Typhimurium (ST) is a major foodborne pathogen in poultry. However, it has been observed that a nutraceutical derived from the olive oil production, fermented defatted "alperujo" (FA), was able to reduce ST caecal colonization in broilers (10.3390/ani10101931). In this survey, we aimed to analyze structural modifications in the bursa of Fabrizio (BF) induced by ST infection in broilers after supplementation with FA.

Methods: Experimental procedures were approved by the Animal Care and Ethics Committee. Twenty 1-day-old broilers, ST-negative, were divided in four groups: G1, negative controls; G2, ST-infected; G3, FA-supplemented (2%); and G4, ST-infected and FA-supplemented. At 7 days-old, groups 2 and 4 were challenged with 3.3×10^5 CFU/mL of ST. Seven days post-challenge (14 days-old), animals were euthanised and necropsied. Ceca content was cultured for ST detection and BF processed for histopathological studies. The area of BF lymphoid follicles (μm^2) was measured using an imaging processing program (Leica App. Suite). In each chicken, 5 follicles were random selected and two diameters (d, µm) measured. From the diameters the area (A, μm^2) was calculated using the formula $\pi*(d1/2+d2/2)^2/4$.

Results: ST was detected from G2 but not from G4. Mean area of BF lymphoid follicles was higher in G3 (228.09 ± 51.57 μm^2), followed by G1 (201.46 ± 83.96 μm^2), G4 (131.55 ± 31.86 μm^2) and G2 (117.85 ± 21.04 μm^2).

Conclusions: FA supplementation may contribute to stimulate BF and prevent ST cecal colonization, which may aid to enhance immune response against ST infection in poultry.

Funding: This project is supported by the One Health EJP

[P125] PREPAREDNESS EXERCISE REVEALED GOOD READINESS FOR OUTBREAK INVESTIGATION IN FINLAND – OUTBREAK COMMUNICATION COULD BE IMPROVED

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Aim: In Finland, municipal investigation groups are responsible for outbreak investigations locally. We arranged an exercise to test the preparedness of the groups to investigate foodborne outbreaks.

Methods: Within one week, we provided municipal environmental health units case-related information daily as the exercise progressed. Units responded measures taken via Webropol survey. We scored the measures taken to identify and control the outbreak and the timeliness of the measures (maximum score = 29) and did descriptive analysis of the survey data.

Results: 42/62 (68%) environmental health units responded. Of these, 55% were responsible for control of 1000-2500 food premises and 50% had 200-400 food premises/ food control person-years. The median preparedness score was 15 (range 8-23); score did not correlate with units' food control person-years. Patient, food or water sampling was organized by all units. Within two days, 82% of the units suspected the source and 33% the causative agent planned in the exercise scenario. Of the units, 30% did not communicate with the public about the outbreak within 3 days.

Conclusions: Most participating environmental health units had good preparedness for outbreak investigations while outbreak communication could be improved. Preparedness exercises enable practicing and maintaining adequate competence at municipalities with sporadic outbreaks.

[P126] FOOD SURVEILLANCE DATA ON CAMPYLOBACTER AND SALMONELLA IN SPAIN (2016-2018): SPATIAL VISUALIZATION AND DESCRIPTIVE ANALYSIS.

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Aim: *Campylobacter* and *Salmonella* are the first and second common food-borne pathogen in humans in the European Union.

In 2018, the number of confirmed cases of human campylobacteriosis and salmonellosis in the EU were 246,571 and 92,000 respectively. In Spain, where both are notifiable diseases, 18,411 and 8,872 of campylobacteriosis and salmonellosis cases in humans were reported in 2018.

In general, campylobacteriosis is associated with the consumption of raw or undercooked meat and meat products, mainly from poultry, but also from domestic ungulates. It can also be transmitted by consumption of raw milk or dairy products without heat treatment, fish and fish products, and raw fruits and vegetables. On the other hand, contaminated water or ice can also be a source of infection.

Salmonellosis is fundamentally associated with the consumption of eggs and raw or undercooked egg-based products, raw or undercooked meat, especially poultry, as well as milk and dairy products not subjected to treatments that eliminate Salmonella; contaminated water, raw fruits and vegetables.

Monitoring of Campylobacter in the EU along the food chain is conducted during the primary production stage (farm animals and their feed), processing (slaughterhouses and cutting plants) and post-processing (wholesale, retail and catering) stages.

In Spain, data on food come from ad hoc official sampling in the context of national monitoring and surveillance programs managed by the Ministry of Health. Official inspections and audits of food establishments are performed by the competent authorities of the 17 Autonomous Communities and the cities of Ceuta and Melilla, coordinated by the Spanish Agency for Food Safety and Nutrition (Ministry of Consumer Affairs). Data also includes analysis on food products. In this work, the official control results from tests for Campylobacter and Salmonella on food for the period 2016 and 2018 are analysed.

Methods: Spatial descriptive analysis

Results: Results (percentage of positive samples) at Autonomous Community level (NUT 2) are displayed in a chloropletic map. Statistics related to major food categories (meat and meat products, milk and milk products) and occurrence at each food chain stage are also reported.

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[P127] RISK FACTORS ASSOCIATED WITH PROLONGED CONVALESCENT EXCRETION OF HUMAN NON-TYPHOIDAL SALMONELLA

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Aim: The aim of the study is to investigate host and pathogen specific risk factors associated with long term shedding of human non-typhoidal Salmonella infections in Norway, to inform infection control and preventive measures.

Methods: All Salmonellosis cases reported to the national surveillance system in 2019 were invited to the study. Participants submitted a stool sample and questionnaire five weeks post-initial sample. We cultured all samples and genome sequenced isolates to identify serotype, virulence- and antimicrobial resistance. We analysed data by descriptive statistics, measures of association and univariable logistic regressions, using two levels of 'length of shedding'; i) Long term shedding (LTS), positive sample at ≥ 5 weeks after initial sample, and ≤ 10 allelic difference, and ii) Short term shedding (STS), negative sample at ≥ 5 weeks.

Results: Data from 273 study participants were included in this analysis. 23.4% (68/273) of the study participants were categorized as LTS and 70.7% (205/273) were categorized as STS. Stratified by Serotype we observed that of the 22 S. Agbeni or Bron isolates 45% (10/22) were LTS in contrast only 21% (6/28) for S. typhimurium or 19% (21/109) for S. enteritidis. Associations were found between the length of shedding and Abdominal pain (p. 0,039) and Diarrhea (p. 0,02) 5 weeks p.i. and Age group 18- 46 (p.0.02).

Conclusions: We found a higher-than-expected proportion of LTS compared to current literature. The small sample size affected our ability to detect further associations. More research is needed to investigate and substantiate the identified associations.

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References:
Information about MoMIR project: https://www.fhi.no/studier/salmonellabaererskap/ https://onehealthejp.eu/jrp-momir/

[P128] GENOMIC COMPARISONS OF EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI (EXPEC) ST73 FROM BRAZILIAN BROILERS AND HUMANS

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Aim: Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a globally distributed pathogen with uropathogenic *E. coli* (UPEC) and sepsis associated *E. coli* (SEPEC) pathotypes causing human disease, while avian pathogenic pathotype (APEC) severely impact poultry. Given the similarities between certain APEC from poultry/meat products and human ExPEC, several studies suggest that APEC may act as a reservoir for human infections. ExPEC sequence type 73 (ST73) is increasingly implicated globally in urinary tract infections and sepsis, but its role in zoonotic disease is less understood.

Methods: We WGS and analyzed the genotypic contents of ten APEC and fourteen UPEC ST73 isolates from Brazil and employing core-genome SNP phylogeny, compared them to a global collection of 542 publicly available ST73 sequences from animal and human sources.

Results: Brazilian ST73 isolates harbored virulence factors frequently associated with UPEC/SEPEC isolates such as *sfa*, *cnf1*, *vat*, *usp*, *hly*A, *mal*X, and iron acquisition and protectins/serum resistance systems while lacking common APEC markers. Analysis of the relatedness of the isolates show close clustering of the Brazilian APEC and UPEC isolates with intermingling with other human and animal worldwide isolates.

Conclusions: We report highly similar ST73 APEC and UPEC in Brazil which also display close relatedness to international UPEC/SEPEC isolates. This suggests a potential for zoonotic transfer of APEC and other ExPEC, pointing in this case, to an anthroponotic route of transmission that could lead to spill-over back to humans from poultry products. These results should be further investigated using prospectively sampled ST73 APEC and employing experimental infection models.

[P129] MOLECULAR DETECTION OF ECHINOCOCCUS MULTILOCULARIS IN FAECES OF RED FOXES USING DIFFERENT DNA EXTRACTION METHODS

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Aim: The aim of the study was to compare the effectiveness of two different DNA extraction methods together with two different PCR protocols in detecting the genetic material of *Echinococcus multilocularis* in the feces of naturally infected foxes.

Methods: DNA extraction was perform for all stool samples with two commercial kits (described as Z and Q). PCR was performed using two different PCR methods: nested PCR and multiplex PCR, for all DNA izolates. Each DNA sample was tested in undiluted and tenfold diluted variant in repetition. Internal control was added to one of two repeated samples, to verify the occurrence of inhibition. Sedimentation and counting technique (SCT) results were treated as reference method.

Results: From 48 samples, 35 were positive in SCT. Nested PCR showed the presence of *E. multilocularis* DNA in 14 (after Z isolation) and 16 (after Q isolation) stool samples in total, 40.0% and 45.7%, respectively (from those with SCT positive result). In multiplex PCR, 19 samples (Z) and 17 (Q) gave positive results, 54.3% and 48.6%, respectively. Twelve SCT positive samples did not give a band specific for *E. multilocularis* DNA in any of the PCRs applied.

Conclusions: Both of the extraction methods showed similar effectiveness in detection of *E. multilocularis* DNA; coped with inhibitors. However, investigation revealed comparatively low sensitivity in field samples from naturally infected foxes, what was presumably related with degradation of genetic material in feaces. This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme, under grant agreement number 773830: One Health European Joint Programme (MEME project; https://onehealthejp.eu/jrp-meme/)

[P130] FACTORS ASSOCIATED WITH SALMONELLA DETECTION IN THE FRAME OF NATIONAL CONTROL PLANS (NCP) IN BREEDING AND LAYING HEN FLOCKS IN SPAIN

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Aim: National Control Plans (NCP) are carried out annually in Europe in poultry flocks for *Salmonella* control [1]. Significantly higher levels of *Salmonella* are reported in samplings carried out by competent authorities (CA) compared with food business operators (FBOp) for programmes requiring separate reporting (broiler and turkey), but reasons behind these differences are not well understood [2]. Here, we conducted a study to assess if this trend was also present in breeding (BF) and laying hen (LHF) flocks in Spain while accounting for the effect of other variables.

Methods: Samplings conducted in LHF (n=66.808), and BF (n=223.351) in 2012-2019 in Spain were included in the study. Temporal and spatial trends in the distribution of positive/negative samplings were explored, and the association of *Salmonella*/target serovars positive results with available variables (year, season, location, sample type, etc.) in addition to sampler (FBOp/CA) was analyzed through uni and multivariable models.

Results: The proportion of positive samples decreased at first in both LHF and BF, and stabilized or increased after 2014 and 2016 respectively, although the contribution of target serovars to this change varied with the host. Several variables were associated with an increased probability of positive results in the samplings, including the sampler but also others such as sample type and region.

Conclusions: Significant differences in the proportion of positive samplings associated with the sampler (FBOp/CA) were also observed in both LHF and BF even after the effect of other covariables was accounted for.

References:

F. Boelaert, G. Amore, Y. Van der Stede, and M. Hugas, “EU-wide monitoring of biological hazards along the food chain: achievements, challenges and EFSA vision for the future,” Current Opinion in Food Science, vol. 12. Elsevier Ltd, pp. 52–62, Dec. 01, 2016, doi: 10.1016/j.cofs.2016.08.004.

EFSA, “The European Union One Health 2019 Zoonoses Report,” EFSA J., vol. 19, no. 2, Feb. 2021, doi: 10.2903/j.efsa.2021.6406.

Funding: Work partially supported by the ADONIS research project funded through the One Health European Joint Programme by the EU’s Horizon-2020 Research and Innovation Programme (grant 773830) and the Spanish Ministry of Agriculture, Fisheries and Food.

[P131] IDENTIFICATION OF ECHINOCOCCUS GRANULOSUS SENSU LATO AT SPECIES LEVEL BY TWO SEQUENTIAL PCR STEPS

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Aim: The zoonotic tapeworm *Echinococcus granulosus sensu lato* (*s.l.*) represents a species complex encompassing multiple causative agents of cystic echinococcosis. Eight genotypes, grouped in five species, are currently recognized within this species complex. Here we present a molecular method that first identifies the common *E. granulosus sensu stricto* (*s.s.*) (genotypes G1 and G3) based on a PCR-RFLP assay, and can further identify the remaining species based on a multiplex PCR (mPCR) assay.

Methods: The method can be applied on DNA extracted from parasitic cyst material of human and animal origin, preserved in ethanol or frozen. First step identifies the common *E. granulosus s.s.* based on a PCR-RFLP assay. A COX1 PCR fragment is amplified, then each product is digested with AluI enzyme producing bands specific for *E. granulosus s.s.*; uncut products are submitted to a multiplex PCR to identify the remaining species. The method was applied both on reference material (*E. granulosus*, *E. multilocularis* and *Taenia* spp.) and on a panel of 65 *Echinococcus* field samples.

Results: Each reference material was correctly identified. Fifty over 65 samples (77 %) showed the two bands pattern expected for *E. granulosus s.s.* The remaining samples were consequently analysed by mPCR, and showed the pattern expected for the *E. ortleppi and E. canadensis* (G6/G7 and G8/G10). To corroborate these results 39 samples were sequenced confirming their identity in all cases.

Conclusions: The method has been developed and validated at the European Union Reference Laboratory for Parasites (EURLP), according to the ISO/IE 17025.

Funding: This project is funded by One Health EJP

[P132] NATIONWIDE FOOD RECALL: STARTING POINT FOR THE ESTABLISHMENT OF AN AEROMONAS LABORATORY IN GERMANY

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Aeromonas bacteria are ubiquitously distributed in aquatic ecosystems and occasionally found in livestock. Some species can cause human intestinal and extraintestinal infections.
In Germany, this bacterial genus attracted considerable attention at the end of 2019, as potentially pathogenic aeromonads were discovered in processed, ready-to-eat animal foods in retail outlets. In order to reduce possible risks for public health, the affected food products were withdrawn from the market in a nationwide campaign. At the request of the German Federal Institute for Risk Assessment (BfR), the suspected *Aeromonas* isolates were sent to the BfR and analyzed using a broad spectrum of analytical methods ranging from biochemical to whole genome sequence-based approaches. Evaluation of all results showed for both isolates that they belong to the so far poorly characterized environmental species *Aeromonas rivuli*, for which information on potential for human pathogenicity is so far unavailable.
This investigation revealed two findings: First, there are no simple standardized detection methods for *Aeromonas* spp. diagnostics. Second, it is not known which of the previously less studied *Aeromonas* species should be classified as potential food pathogens.
Against this background, a laboratory for study of *Aeromonas* was set up at the BfR. Here, first insights into the objectives of the work of this laboratory are given.

[P133] RRISK SHINY APP FOR RISK ASSESSMENT

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Aim: Rrisk Shiny is a web-based tool for risk assessment using Monte-Carlo simulation and helps to build quantitative risk models via a clearly and well-structured graphical user interface. Rrisk Shiny consists of several modules, which together compose a framework for probabilistic risk assessment.

Methods: A special functionality of Rrisk Shiny is the documentation of uncertainties in risk models with a traffic light system. All data and models remain with the user and are not stored on the Shiny Rrisk server. Both the program itself and the program code are freely available. Rrisk Shiny is an object oriented implementation R programming language of both original code and R packages.

Results:
The following features characterize the program.
User-friendly GUI provides

- Using the functions of R packages without programming
- Model building (variables, parameters, equations, results)
- One dimensional and two dimensional Monte-Carlo simulation
- Data fitting
- Model visualization
- Schema for the assessment of uncertainties (EFSA Guidance)
- Transparency through identity between model and its documentation
- Auto-reporting
- State-of-art risk modelling methodology through rich functionality (resampling, bootstrapping, model network graph)
- Uncertainty analysis
- Reproducibility, version control and portability are ensured
- Good practice of complete and consistent model documentation is embedded in the model development
- Transparency and congruence is ensured through identity between the model and its description
- Model export in JSON and RDA formats

[P134] THE ONE HEALTH EUROPEAN JOINT PROGRAMME OUTCOME INVENTORY (OHOI): A DATABASE FOR WIDE DISSEMINATION OF RESULTS AND UPDATES FROM THE CONSORTIUM

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Aim: To achieve the best use of outcomes of the One Health European Joint Programme (EJP), they have to reach a broad and diverse audience. The general and targeted dissemination efforts cannot reach all the stakeholders nor disseminate all the output produced. To close this gap, the One Health EJP Outcome Inventory (OHOI) is in place.

Methods: Outcomes and updates of the One Health EJP's 24 Joint Research Projects, 5 Joint Integrative Projects, PhD projects, and overarching activities, are catalogued in the OHOI. Information is regularly gathered, summarized, validated, and uploaded online in the relational database, which also includes features for user-friendly navigation. The OHOI is publicly available to any interested party, supporting dissemination as well as collaboration within and with the One Health EJP. To support networking, the OHOI includes contact information of the actors involved. Linking with the projects' Data Management Plans is planned.

Results: The OHOI is regularly updated and expanded. As of March 2021, it contains 77 outcomes (25 databases, 44 tools and 8 strain collections) and 787 updates.

Conclusions: The activities of the One Health EJP consortium are numerous and diverse. While its results are made readily available for policy- and decision-making through targeted dissemination, the OHOI adds value by displaying the full range of outcomes to a wide audience, increasing the current and future impact of the One Health EJP.

References:
One Health EJP Outcome Inventory: https://onehealthejp.eu/outcome-inventory/

Outcomes in the OHOI: https://c1abo859.caspio.com/dp/e05d7000137f7c9dd846442f83fc

Updates in the OHOI: https://c1abo859.caspio.com/dp/e05d70001b9da2f2fb0a4818b5f8

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[P135] ONE HEALTH EUROPEAN JOINT PROGRAMME SCIENCE TO POLICY TRANSLATION THROUGH EFFECTIVE INTERACTION WITH STAKEHOLDERS

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Aim: Mechanisms for science to policy translation aim at maximising the impact of the One Health European Joint Programme (EJP) scientific outcomes and results.

Methods: In One Health EJP, efficient science to policy translation is achieved by complementing broad dissemination activities by active dialogue with stakeholders and by targeted support and dissemination. One Health EJP systematically collects and analyses stakeholders' needs, and disseminates its outcomes accordingly. A number of tools facilitate the interaction with stakeholders: Meetings are organised on a regular basis with the Stakeholders' Committee (including ECDC, EFSA, EEA, EMA, FAO, OIE and WHO-EURO), and with national stakeholders. Targeted and thematic reports address specific needs and provide updates of the work done. The One Health EJP Outcome Inventory provides structured information on outcomes and updates of the consortium's projects. Additional support is provided, for example, by organizing thematic workshops.

Results: One Health EJP partner institutions have a mandate from their national or regional authorities, and the activities of the consortium follow a prioritized strategic research agenda that addresses collected needs of a wide range of stakeholders at the national, European, and international level. Outcomes of the consortium are disseminated to address the identified needs. Additional strategies have offered timely support to the stakeholders as new needs have emerged.

Conclusions: The efficient science to policy translation of One Health EJP, from gap-driven research to FAIR outcomes, is proving to be an example of impactful use of research funding.

References:
One Health EJP Outcome Inventory: https://onehealthejp.eu/outcome-inventory/

Thematic Reports: https://onehealthejp.eu/science-to-policy-translation/

Strategic Research Agenda: https://onehealthejp.eu/wp-content/uploads/2018/12/One-Health-EJP Strategic-Research-Agenda.pdf

Funding: This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

[P136] PREVALENCE AND CHARACTERIZATION OF SALMONELLA SPP. AND PATHOGENIC ESCHERICHIA COLI IN FOOD-PRODUCING ANIMALS IN PORTUGAL

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Aim: To determine the prevalence and characterize *Salmonella spp.* and pathogenic *Escherichia coli* in food-producing animals from different farms located in mainland Portugal, as part of OH-EJP DISCOVER.

Methods: In January 2021, 74 fresh faecal samples were obtained from cattle (N=40), and pigs (N=34), from four farms. Isolation was performed in selective and non-selective medium, followed by identification of virulence genes of *E. coli* by multiplex PCR, and *Salmonella* serotyping. Antimicrobial susceptibility of *Salmonella* isolates was tested. PCR for the detection of *mcr* genes was also performed. Whole-genome sequencing and *E.coli* susceptibility testing is in progress.

Results: Regarding cattle, no *Salmonella* was isolated from the faecal samples, whereas 35 samples (87.5%) were positive for *E.coli*. Only two isolates (5.7%; 2/35) were pathogenic, exhibiting *vtx2*, and *vtx1+vtx2*.

In pigs, *Salmonella* was detected in 20.6% (7/34), of which 85.7% were *S.*4,5:i:- (6/7) and 14.3% *S.*Rissen (1/7), while 32 samples were positive for *E.coli* (94.1%). Only two isolates (6.3%; 2/32) were positive for virulence genes, in this case *eae*. Curiously one sample was positive for EPEC and also for *S.*4,5:i:-.

Preliminary results for AMR testing showed that none *Salmonella* isolate harbours *mcr* genes, one *S.*4,5:i:- was resistant to tetracycline, other to tetracycline and sulfamethoxazole, and four to tetracycline, sulfamethoxazole, and ampicillin. *S.*Rissen was resistant to nalidixic acid, pefloxacin, trimethoprim, sulfamethoxazole and azithromycin.

Conclusions: Multidrug-resistant *Salmonella* and pathogenic *E.coli* can be transmited through a variety of sources, namely food-producing animals. The decrease of human infections will only be achieved with a OneHealth approach.

Funding: This work is supported by One Health EJP funding, namely by the JRP DiSCoVer

[P137] DUCKS AS A RESERVOIR OF RESISTANT ESCHERICHIA COLI

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Aim: Antimicrobial resistance (AMR) in poultry remains a source of concern as it may pose a risk to public health *via* food of animal origin. Comparing to other poultry species, studies of ducks in the above-mentioned area seem to be insufficient. Thus we aimed to evaluate the AMR occurrence in *Escherichia* (*E.*) *coli* isolated from ducks.

Methods: The methods used in official AMR monitoring were applied to investigate the AMR status of *E. coli* from boot swabs collected at 152 duck farms in Poland. The samples were screened for commensal, cephalosporin- and carbapenem- resistant *E. coli*. Isolates were tested for antimicrobial susceptibility with microbroth dilution method (Sensititre EUVSEC plates; TREK Diagnostic Systems) with respect to 9 antimicrobial classes: beta-lactams, quinolones, phenicols, aminoglycosides, folate-path inhibitors, tetracyclines, polymyxins, macrolides and glycylcyclines. Epidemiological cut-off values were applied as interpretation criteria. Whole Genome Sequencing (WGS) of a subset of cephalosporin-resistant strains (n=25) enabled an in-depth insight into specific resistance mechanisms.

Results: A total of 160 *E. coli* were isolated. Over 81% of strains were found resistant (n=131). Of all antimicrobials assessed ciprofloxacin, ampicillin and tetracycline resistance dominated (66.3%, 58.1%, 50%, respectively) followed by folate-path inhibitors (40.6% sulfamethoxazole and 34.4% trimethoprim) and nalidixic acid (32.5%). A lower percentage of resistance was observed for chloramphenicol (18.1%), and cephalosporins (16.9%). Four strains (2.5%) were resistant to gentamicin. No carbapenem-resistant *E. coli* were noted. WGS revealed numerous determinants including among others: $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$, *qnrS1*, *qnrB19*, *aac(3)-IIa*, *aph(3')-Ia*. One of the tested strains carried *mcr-1* that encodes colistin resistance although it was not confirmed phenotypically.

Conclusions: Our study shows that ducks constitute a meaningful reservoir of *E. coli* carrying multiple resistance determinants including those of public health concern.

Funding: Laboratory study was performed within task 17 of Multiannual Research Project (2019 – 2023). Current analysis was partially supported by Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards - One Health EJP GA No 773830.

[P138] IMPACT OF GENETIC VARIATION OF 200 LISTERIA MONOCYTOGENES ISOLATES ON GROWTH IN PRESENCE OF PRESERVATIVES

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Aim: *L. monocytogenes* is a ubiquitous bacterium, but genetic differences along the farm-to-fork chain is expected due to stress exposure. The purpose was to investigate whether genetically different strains of *L. monocytogenes* responded differently to preservatives and pH.

Methods: Strains from the ListAdapt library were used. All strains were grown singularly in BHI broth at 12°C with sodium lactate (0-4000 ppm) and/or acetate (0-1000 ppm) at pH 4.5 or 7. The growth was followed by measuring the optical density (OD). The clonal complex groups and the phylogenic tree were derived from DNA sequences and analysed with bioinformatic methods.

Results: No differences were observed between isolates at pH 7. At pH 4.5, however, different growth patterns and sensitivities to preservatives, in particular to sodium acetate, were seen. The most sensitive strains were distributed in the phylogenic tree across lineages and CC groups of *L. monocytogenes*, as well as between strains isolated from food, animals and nature.

Conclusions: Different growth patterns and sensitivity to preservatives were seen among strains, but only at low pH. No clustering of sensitive strains and CC groups or niches of isolation were observed. Other genetic characteristics for the sensitive strains will be searched for.

The results indicate that genetic variation needs to be considered when assessing the safety of foods with low pH, and also in selection of suitable strains for challenge studies and development of predictive models for low pH foods.

[P139] PRELIMINARY DESCRIPTION OF BIOSECURITY PRACTICES RELATED TO IMPORTING PIG AND SEMEN ONTO EUROPEAN PIG FARMS.

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Aim: A description of biosecurity measures related to the import of pigs and semen onto European pig farms, to inform which measures were common, and to describe differences between farm types.

Methods: Between 18-50 pig farms were selected from each of 10 European countries. These included 1) fattening, 2) breeding (which supply pigs that will go to farms to finish them for slaughter) and 3) farrow-to-finish (F2F) units. Small holdings and Specific Pathogen Free farms were excluded from the study. The selection of farms was (where possible) to represent the most common sizes and types of farming in each country. For each farm an interview was completed to collect biosecurity data, according to a standardised questionnaire of 56 questions on farm biosecurity. Only the eight questions related to imports of pigs or semen are presented here.

Results: At present, 90 out of a planned 300 farms have submitted data and these preliminary findings are reported here. The population included 47 F2F units, 15 breeders and 28 finishers. Most F2F and breeder farms imported semen from boar studs (72.6%), with a minority using their own boars (12.9%) or their own boars as well as boar studs (14.5%). Most farms imported breeder pigs from a single source, with only 10.5% using multiple sources. Generally, the semen and pigs came from sources with unknown *Salmonella* and Hepatitis E virus (HEV) status. However, breeder farms were more likely to import semen from sources with equal or higher *Salmonella* status than the other farm types. Quarantine for both gilts and boars (if applicable) was used by breeder farms (88.9%), with a duration range of 28-70 days, whereas a lower proportion of F2F farms used quarantine for these pigs (68.0%), with a range of 0-150 days.

Conclusions: The preliminary results identify some issues with imports of semen and pigs, with source farms generally being of unknown *Salmonella* and HEV status and quarantine not always applied on a minority of the farms. These results will be joined to faecal sample results to allow analysis of which biosecurity measures were significantly associated with higher or lower risk of *Salmonella* and HEV.

Funding: Funding: This study is funded by the BIOPIGEE project

[P140] PET OWNERSHIP AND CHRONIC DISEASE: A SURVEY EXAMINING THE RELATIONSHIP

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Aim: Pets are an important part of many peoples' lives and this has been especially true during the COVID-19 pandemic. Anecdotally, pets improve their owner's mental and physical health but limited evidence is available to support these statements. This study examined the impact of pet ownership on people with chronic disease compared to those without pets. For this study, pet ownership was limited to dogs and cats.

Methods: A mixed-mode survey was conducted in the US across four regions of the country and questions focused on general health and life satisfaction.

Results: A total of 1,520 people with chronic disease, 796 pet owners and 724 non-pet owners, completed the survey. Pet owners reported fewer days than non-pet owners that their physical health ($p = 0.00$) or mental health ($p = 0.025$) was "not good". Pets owners were significantly less likely than non-pet owners to report their physical or mental health prevented them from performing their normal daily activities ($p = 0.00$).

Conclusions: In general, pet owners reported overall better health and better social and emotional support than non-pet owners. These results provide support that pet ownership may provide both physical and mental health benefits to people with at least one chronic disease when compared to those without pets.

Funding: This research was funded with a grant from Maddie's Fund.

[P141] PET OWNERSHIP AND MENTAL HEALTH: A REVIEW OF THE LITERATURE

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Aim: Pets play an important role in the lives of people and have been especially important during the COVID-19 pandemic. Anecdotally, pet ownership can lead to improved physical and mental health for owners, but scant research is available validating these claims.

Methods: This study aimed to review the peer reviewed literature to better describe the body of knowledge surrounding the relationship between pet ownership and mental health.

Results: After title review, abstract review and then full article review, 54 articles were included in the final analysis. Of the 54 studies, 18 were conducted in the general population, 15 were conducted in an elderly population, 8 were conducted in children and adolescents, 9 focused on people with chronic disease, and 4 examined a specific unique population. Forty-one of the studies were cross-sectional, 11 were prospective longitudinal cohort, and two were other study designs. For each of the articles, the impact of pet ownership on the mental health of owners was divided into four categories: positive impact (n = 18), mixed impact (n = 17), no impact (n = 14), and negative impact (n = 5).

Conclusions: Among the reviewed articles, there was much variation in population studied and study design, and these differences make direct comparison challenging, however, when focusing on the impact of pet ownership on mental health, the results were variable and not wholly supportive of the benefit of pets on mental health.

Funding: This research was funded by a grant from Maddie's Fund.

[P142] GENOTYPING AND VIABILITY ASSESSMENT OF TOXOPLASMA GONDII PARASITES DETECTED IN MEAT PRODUCTS

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Aim: Raw or undercooked meat with tissue cysts containing bradyzoites, is considered a major source of human Toxoplasma gondii infections in Europe, however data concerning this issue in Poland are still insufficient. The aim of study was to determine the prevalence of DNA and live T. gondii parasite in raw meat products retailed in Poland. The molecular characteristics of detected DNA were also performed.

Methods: Samples (50 g) of raw cured bacon, sausages, ham and minced meat (871 samples) were digested by pepsin solution, followed by the DNA isolation. Nested and Real-time polymerase chain reaction (PCR) was performed based on the amplification of B1 fragment gene of T. gondii. For selected PCR positive samples, multilocus RFLP PCR was performed. To assess the viability of T. gondii, isolation assays by cell culture and bioassay on mice were performed.

Results: Among 871 examined samples, 54 (6%) were PCR positive. The highest percentages of positive results were found for samples from Pomorskie (12%) and Warmińsko-Mazurskie, lowest from Lubelskie regions of Poland. The percentages of positive results for particular types of meat products ranged from 1.5% to 9%. RFLP analysis showed mostly the alleles of clonal type II (46%) and III (34%), the combinations of types alleles at different loci were also found (20%). The viable T. gondii was isolated from 17 samples.

Conclusions: Detection of T. gondii DNA and a live parasite in 6% and 2% of the tested raw meat products samples, respectively, indicate a real threat to the health of consumers.

[P143] AN EXPLORATION OF ANTIMICROBIAL PRESCRIBING PRACTICE AND PRESCRIBING INTENTION OF VETS FOR LIVESTOCK AND GPS IN RURAL PRACTICES IN SCOTLAND 2019

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Aim: This aim of this study is to explore antimicrobial prescribing practice and prescribing intention of vets for livestock and GPs in several overlapping rural areas, to examine promoters and barriers to optimal AM prescribing, and to explore if there are lessons which can be transferred between the two groups of prescribers.

Methods: The study used semi-structured telephone interviews based on the following predetermined set of open questions. A small group of GPs and Vets who were part of the initial scoping study were invited to participate in the qualitative study to investigate attitudes to prescribing of antibiotics.

Results: Prescribing is a highly complex decision rather than a simple binary decision. GPs increasingly deal with co-morbidities which can make prescribing choices very complex on an individual level. Vets balance the needs and circumstances of the farmer with the health needs of livestock and the needs of one animal within the context of herd or flock health. GPs may be influenced by the psycho-social, remote location and economic needs of their patients whereas vets may be influenced by the economic value of their patients and by their business and personal relationships with farmers. Adherence to drug administration and practicalities of dosing regimens can influence prescribing practice of both GPs and vets.

Conclusions: Education of the public and farming communities about antimicrobial resistance would support vets and rural GPs in their efforts to reduce their levels of prescribing of antimicrobials and to engage with patients and farmers in accepting alternatives.

[P144] INVOLVEMENT OF THE SOCIAL SCIENCES SECTOR IN ONE HEALTH NETWORKS AND COVID-19 RESPONSE

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Aim: We explored the data from a larger global study to describe the participation of the social sciences sector in One Health Networks (OHNs) and involvement in COVID-19 response.

Methods: This work is part of a larger cross-sectional study, conducted in 2020, that included a global online survey on OHNs and COVID-19 response. The 16-question survey focused on participation in OHNs and involvement in the COVID-19 response. We analyzed the results of those survey respondents who indicated they were from the social sciences sector.

Results: Altogether, 79 of the 1050 respondents of the global survey were from the social sciences sector. Sixty (76%) of the 79 indicated that they were part of an OHN, and being part of an OHN was positively associated with involvement with the COVID-19 response (odds ratio 4.5, 95% confidence interval: 1.5-13.9).

Conclusions: While the number of respondents from the social sciences sector was small, the results suggest that their participation in an OHN was beneficial for their involvement in response to the pandemic. With greater understanding of the participation of the social sciences sector in OHNs and involvement in the COVID-19 response, OHNs could develop targeted and inclusive activities involving social scientists in efforts to build workforce capacity in One Health.

Funding: This work was supported by the One Health Commission and funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health.

[P145] LAMP POINT-OF-CARE TEST FOR ETEC DETECTION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING

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Aim: Post-weaning diarrhea (PWD) caused by enterotoxigenic Escherichia coli (ETEC) accounts for the majority of antibiotic use in pigs. The aim of this study was to validate a point-of-care test based on colorimetric loop-mediated isothermal amplification (LAMP) for on-farm detection and susceptibility testing of ETEC to antimicrobials important for PWD treatment, namely neomycin and tetracycline.

Methods: Primers for the LAMP reaction were designed to target the predominant fimbrial (F4 and F18) and toxin genes (STb and LT) in ETEC, and genes conferring resistance to neomycin (aph(3')-la, aph(3')-lb) and tetracycline (tetA and tetB). Performance of the LAMP test (65 °C for 30 min) was evaluated on 442 clinical E. coli isolates obtained from Danish pigs in 2020 using standard PCR as the gold standard.

Results: LAMP results were in 100% agreement with PCR results and showed that 186 out of 442 E. coli isolates (42.1%) were ETEC, being positive for the presence of at least one toxin. More than 80% of ETEC strains (152/186) were either F4- or F18-positive. Neomycin and tetracycline resistance genes were detected in 21.5% and 60.0% of E. coli isolates, respectively.

Conclusions: We propose a cost-effective point-of-care test that can be used by farmers and veterinarians for rapid diagnostics and targeted treatment of PWD caused by ETEC. The test contributes to prudent antimicrobial use by reducing empiric antibiotic use that may result in treatment failure and overuse of critically important antimicrobials. Next, the test will be evaluated first in diagnostic specimens and then in a farm setting.

Funding: We acknowledge the financial aid from the Danish Veterinary and Food Administration (Fødevarestyrelsen).

[P146] CONTACT WITH CATTLE ASSOCIATED WITH INCREASED CRYPTOSPORIDIOSIS IN HUMANS IN FINLAND

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Aim: In Finland, cryptosporidiosis has increased over 20-fold since the early 2000's. We launched a case-control study and analyzed *Cryptosporidium* species to identify sources of the increase and to apply appropriate control measures.

Methods: Case was a person with cryptosporidiosis notified to Finnish Infectious Disease Register 1.7.-31.12.2019. Questionnaire was sent to cases and controls matched by age, gender and hospital district. The exposures with a p-value <0.05 in univariate analysis were included in multivariate analysis. *Cryptosporidium* species in patient samples was determined by PCR.

Results: Of cases, 45% (115/254) and 16% of controls (239/1516) answered the questionnaire. *Cryptosporidium parvum* was identified in all (78/78) samples of cases answering questionnaire. 68% of cases and controls were female. Cases' median age was 37.5 years (5–59 years) and controls' 34 years (1–61 years). Mean duration of symptoms was 12 days (4– >26 days). Most common symptoms were diarrhoea (97%), weakness (82%) and nausea (77%). Of cases, 30% received intravenous fluids and 10% were hospitalized. Cryptosporidiosis was associated with contact with cattle (OR 82, 95% confidence interval (CI) 22–300), having a family member with gastroenteritis (OR 27, 95% CI 4.6–160) and spending time at own vacation home (OR 16, 95% CI 4.2–60).

Conclusions: *C. parvum* is most common cause of cryptosporidiosis in Finland. Cryptosporidiosis is an important occupational disease at cattle farms. Guidelines to control occupational exposure were created and information on cryptosporidiosis and control measures shared to health care, veterinary and farm work professionals.

[P147] INCREASING TREND IN CAPNOCYTOPHAGA CANIMORSUS NOTIFICATIONS IN FINLAND, 2000-2019

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Aim: Zoonotic bacterium *Capnocytophaga canimorsus* causes meningitis, endocarditis, sepsis and death in humans. Infections are often associated with dog or cat bites, scratches or wound licking. We describe *C. canimorsus* patients' demographics in Finland in 2000–2019.

Methods: Case was a person with *C. canimorsus* isolated from blood or cerebrospinal fluid and notified to Finnish Infectious Disease Register (FIDR) in 2000–2019.We characterized cases according to age, gender, sampling site, case fatality and hospital district. Incidence rate ratios (IRR) were calculated to compare incidences.

Results: In 2000–2019, 343 (annual range 6–39) *C. canimorsus* findings were notified to FIDR (mean annual incidence 3.17 per million). In 2010–2019, incidence increased 3-fold compared to 2000–2009 (IRR 2.66, 95% confidence interval (CI) 2.09-3.38). Of cases, 55% were male. Median age was 58 years (12–94 years). Incidence was 3-fold in 50–69-year olds compared to other age groups (IRR 3.32, 95% CI 2.69–4.11). *C. canimorsus* was mostly detected in blood (98%). Seventeen cases (5%) died. Mean annual incidences in hospital districts ranged from 0.22 to 7.03 per million. Incidence was 1.5-fold in Eastern districts compared to other districts (IRR 1.55, 95% CI 1.19-2.0).

Conclusions: In Finland, *C. canimorsus* infection incidence has increased and is higher, while case fatality is lower, than in reports from other countries. Risk groups need information on how to prevent exposures and act after exposure. Detailed information on sources, risk factors and geographicality are needed to understand *Capnocytophaga* infection epidemiology and to target control measures.

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[P149] GENOMIC ANALYSIS OF ESCHERICHIA COLI ISOLATES FROM RAPTORS IN EURASIA

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Genomic analysis of *Escherichia coli* isolates from raptors in Eurasia
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Aim: Eurasian raptors were examined for the carriage of ESBL and AmpC-producing *Escherichia coli* isolates. Whole genome sequencing (WGS) was used to determine the genetic content and phylogenetic relationships.

Methods: Cloacal samples (n=348) from raptors in Austria, Belgium, Czech Republic, Denmark, Hungary, Ukraine, Russia and Slovakia were cultivated on MacConkey agar with cefotaxime to select for resistant *E. coli.* Susceptibility testing and PCR were conducted to identify ESBL and AmpC phenotypes and genes, respectively, and was followed by WGS.

Results: A total of 45 *E. coli* isolates of various sequence types (STs; 34 different STs) exhibiting ESBL (9.4%, n=348) or AmpC phenotypes (3.4%, n=348) were obtained. All of them carried virulence-associated genes (VAGs) linked to pathogenic *E. coli*. Fifty-nine various antibiotic-resistance genes (ARG) were identified in the selected isolates with a mean of 5.3 ARG/isolate. Common beta-lactamase genes included $bla_{\text{CTX-M-1}}$ (26.6%), $bla_{\text{TEM-1B}}$ (20%), $bla_{\text{CMY-2}}$ (20%) and $bla_{\text{CTX-M-15}}$ (17.7%). Chromosomally-located carbapenem-resistance gene $bla_{\text{OXA-244}}$ was part of an incomplete LE1 prophage in *E. coli* ST93. A rare quinolone resistance *qnrE1* was identified within a multi-drug resistance *E. coli* ST354 on IncHII2-ST3 plasmid. A clonal relation (1-30 SNP difference) was observed between three isolates of ST162 showing similar profiles for ARGs, VAGs and plasmid content and originating from Russian black kites and an imperial eagle in the Czech Republic.

Conclusions: We report the occurrence of ESBL-producing pathogenic *E. coli* in Eurasian raptors and the presence of closely related *E. coli* ST162 isolates sourced from different raptors and locations.

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[P150] IMMUNOMAGNETIC SEPARATION IMPROVES THE ISOLATION OF ESCHERICHIA COLI O55 FROM BOVINE FAECAL SAMPLES

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Aim: To improve the isolation of the Shiga toxin-producing *Escherichia coli* (STEC) serotype O55, which is associated with human infection in Europe, as there is currently no selective isolation method for its detection alone.

Methods: Immunomagnetic separation (IMS) and a real-time PCR (rtPCR) designed by Kirchner *et al*. (2019), were used to isolate *E. coli* O55 from pure bacterial cultures of ten pooled non-*E. coli* O55 serotypes and bovine faecal samples that were spiked with strains of *E. coli* O55. Ten-fold serial dilutions (from 10^9 to 10^1 CFU/ml) of *E. coli* O55 strains were used to spike two sets of the samples before IMS was performed on one set of the spiked dilutions, followed by rtPCR on resultant colonies that grew on CHROMagar ECC agar plates. Additionally, rtPCR was performed on the second set of spiked samples in order to compare the results to the samples treated with IMS.

Results: IMS performed on bacterial cultures of pooled non-*E. coli* O55 serotypes spiked with *E. coli* O55 strains resulted in the detection of 10^3 to 10^9 CFU/ml of *E. coli* O55, which was identical to results obtained from rtPCR alone. However, IMS enabled the detection of 10^3 to 10^9 CFU/ml *E. coli* O55 compared to 10^7 to 10^9 CFU/ml when rtPCR was performed alone on bovine faecal samples.

Conclusions: Performing IMS prior to rtPCR improves the isolation of *E. coli* O55 in bovine faecal samples and will aid diagnostics and surveillance of future outbreaks caused by STEC *E. coli* O55.

References:
Kirchner M, Sayers E, Cawthraw S, Duggett N, Gosling R, Jenkins C, *et al*. A sensitive method for the recovery of *Escherichia coli* serogroup O55 including Shiga toxin-producing variants for potential use in outbreaks. *J Appl Microbiol*. 2019; 127(3): 889-896. Available from doi: 10.1111/jam.14345.

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[P151] THE PREVALENCE AND ANTIMICROBIAL RESISTANCE OF CAMPYLOBACTER SPP. ISOLATED FROM FRESH BROILER CHICKEN MEAT SOLD AT ESTONIAN RETAIL

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Aim: The aim of the present study was to investigate prevalence, counts and antimicrobial resistance of *Campylobacter* spp. in fresh broiler chicken meat of Estonian, Latvian and Lithuanian origin at Estonian retail.

Methods: 399 fresh broiler chicken meat samples were collected between September 2018 and August 2019 from the biggest supermarket retail outlets in Estonia. *Campylobacter* spp. enumeration and detection was performed according to ISO 10272–1:2017. 61 *Campylobacter* isolates were tested against erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin and nalidixic acid for MICs via the VetMIC Camp method (National Veterinary Institute, Uppsala, Sweden).

Results: Fresh broiler chicken meat of Estonian origin is generally free of *Campylobacter*, but the imported broiler chicken meat was often contaminated. Few samples of Estonian origin that were *Campylobacter* positive had *Campylobacter* numbers below the detection limit (<100 CFU/g). While in samples of Latvian origin the presence of *Campylobacter* in high concentration was found in one sample, then in Lithuanian products high *Campylobacter* concentrations were found throughout the study period. *Campylobacter* isolates showed the highest resistance to nalidixic acid (55 strains; 90.2%) and ciprofloxacin (55 strains; 90.2%), followed by tetracycline (35 strains; 57.4%), streptomycin (26 strains; 42.6%) and erythromycin (4 strains; 6.6%). All strains were sensitive to gentamicin.

Conclusions: Fresh broiler meat of Estonian origin is generally free of *Campylobacter*. Fresh broiler meat of Latvian and Lithuanian origin is often contaminated. While *Campylobacter* isolates of Estonian origin samples proved to be sensitive to tested antibiotics, isolates obtained from Latvian and Lithuanian origin samples were resistant to one or more antibiotics.

[P152] INTERCONTINENTAL COLLABORATIONS AND APPLICATIONS OF WHOLE GENOME SEQUENCING FOR CONTROL OF ZOONOTIC MYCOBACTERIUM BOVIS TO PROMOTE GLOBAL-ONE-HEALTH

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Aim: The aim of the present study is to characterize *Mycobacterium bovis* isolates isolated from human-animal interface using whole-genome sequencing (WGS) technology.

Methods: *M. bovis* isolates included in the present WGS study were from Ethiopia, Uganda and Zambia. The *M. bovis* were isolated from bovine, swine and human TB cases. The *M. bovis* isolates were chosen to maximize genetic diversity representing different host, tissue type, geographic location and/or type of farming systems.

Results: Most of the isolates from Ethiopia and Uganda belonged to African clonal complex 2. All the *M. bovis* isolates originated from Zambia were closely related to each other, and were outside the clade of any previously designated clonal complex. Interestingly, some of the Ugandan *M. bovis* isolates were pyrazinamide susceptible and clustered between *M. caprae* and *M. bovis*. Collaborations among researchers form different continents (Africa, Europe and America) is crucial for promoting global-one-health.

Conclusions: The WGS revealed the presence of conserved SNPs signatures among *M. bovis* isolates originated from different geographical regions independent of origin of host. The WGS identified unique pyrazinamide susceptible *M. bovis* isolates.

[P153] LIN-RES PROJECT: GENOMIC ANALYSIS OF LINEZOLID RESISTANT STRAINS ISOLATED FROM HEALTHY ANIMALS AND HUMAN PATIENTS.

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Aim: Linezolid is a last resort antibiotic to fight human infections caused by multi-resistant Gram-positive bacteria such as staphylococci and enterococci. Resistance is caused by point mutations in 23SrRNA or plasmid-borne genes [cfr, cfr(B), optrA or poxtA]. Through the LIN-RES project, 155 linezolid resistant (LR) isolates (150 from diverse healthy food-producing animals and 5 from human patients) were collected in Belgium: 147 enterococci, 7 staphylococci, and 1 Pediococcus pentosaceus. The aim of this part of the project was to characterize genetically these LR isolates and their relatedness.

Methods: LR determinants and genetic organisations surrounding them were determined through whole genome sequencing analysis. The genetic relatedness was assessed through cgMLST analysis.

Results: Among the 155 isolates, all but 4 harboured either optrA, poxtA or cfr or a combination of these genes. All LR isolates lacking LR genes contained 23sRNA mutations. Several different organisations surrounding LR genes were observed. LR genes were often associated with other resistance genes. The cgMLST analysis revealed a large diversity among the isolates.

Conclusions: This study has shown that LR was mainly due to plasmid-borne genes and that LR is present in a large diversity of strains isolated from food-producing animals, with some strains closely related to human isolates, posing a risk to human health. Conjugation analyses will be conducted to confirm the plasmid location of the LR genes and estimate the transfer rates of plasmids carrying LR from this collection of isolates.

Funding: This study is funded by One Health EJP “LIN-RES”

[P154] GONE FISHING! POST-DNA EXTRACTION ENRICHMENT BASED ON SEQUENCE-BASED CAPTURE OF LOW ABUNDANCE CRYPTOSPORIDIUM DNA

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Gone fishing! Post-DNA extraction enrichment based on sequence-based capture of low abundance *Cryptosporidium* DNA

Aim: The food- and waterborne parasite *Cryptosporidium* cause diarrheal disease worlwide. Detection of *Cryptosporidium* on food often relies upon microscopic identification of oocysts, and is complicated by low contamination levels, resulting in under-detection. The complex nature of food samples makes molecular methods uneasy to apply, and standard DNA extraction procedures generate insufficient amounts of DNA from the target organism unless an enrichment step is applied. This will, unless the sample is highly contaminated, result in false negative results. As part of the PARADISE project we aim to develop a post-DNA enrichment method to concentrate specific parasite DNA.

Methods: We have developed a protocol based on hybridization of target-specific biotinylated probes to catch DNA fragments used for species determination (*SSU*) and subtyping (*gp60*) of *Cryptosporidium* spp. Probes and helper probes, to pull out target DNA using streptavidin-coupled magnetic beads have been designed and evaluated. qPCR for the specific markers have been used to evaluate the capture procedure. Sensitivity testing has been performed.

Results: Our capture probe system works well for both markers. We can amplify and sequence target DNA down to 10 oocysts per 1.5 mL sample (*SSU*) and can detect approximately 100X lower spiking amounts compared to DNA isolation using conventional extraction methods (*gp60*).

Conclusions: The generated hybridization capture probes enable detection of low abundance DNA, also in complex samples not extracted with *Cryptosporidium* as a target.

Funding: The PARADISE project is funded by the European Union's Horizon 2020 Research and Innovation Programme, grant agreement No 773830: One Health European Joint Programme.

[P155] WILBR: CONTRIBUTION OF WILD BIRDS TO AMR IN THE ENVIRONMENT AND ON FARM

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Aim: Despite wild birds not being intentionally exposed to antimicrobials, the presence of AMR bacteria is widespread in some wild bird populations. This study aims to explore wild birds as a likely route of transmission of AMR to new environments, including the farm environment, in association with the OHEJP WILBR PhD project.

Methods: Faecal samples were collected from gulls and pigs of five different age classes, over 3 time-points at 12 month intervals on a UK pig farm. Escherichia coli were isolated on antibiotic-free and antibiotic-containing agar plates and underwent whole genome sequencing. Downstream sequencing analysis was carried out to assess the diversity of E. coli strains and characterise the AMR genes, to assess the potential for transmission between gulls and pigs.

Results: In total, 632 E. coli were isolated from pig and gull faeces (n=342 and n=290 respectively). E. coli Sequence Type (ST) 744 (31.5%), 10 (14.2%), 88 (10.7%), and 44 (8.4%) were the most prevalent. Interestingly these were also the only ST types present in both gull and pig faeces. From antibiotic-free plates 44.6% of isolates from pigs harboured 1-12 AMR genes, and 36% of isolates from gulls harboured 1-15 AMR genes. The majority of ST744s were isolated from ciprofloxacin containing plates and harboured multiple AMR genes.

Conclusions: The presence of E. coli strains of the same ST type in both gull and pig faecal, from multiple time points, indicates the persistence of antimicrobial resistance in the farm environment and the possible transmisison of multi-drug resistant E. coli.

[P156] ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF ENTEROBACTERALES ISOLATED FROM MANURE AMENDED GRASSLAND

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Aim: The transfer of antimicrobial resistant bacteria (ARB) from manure to soil has been documented; however little is known about the involvement of the grass phyllosphere in the dissemination of ARB. The aim of this study was to investigate the impact manure application has on the soil and grass culturable microbiome, focusing on the occurrence and resistance profiles of two members of the Enterobacterales: *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae).*

Methods: A field trial was carried out in the summer of 2019 in Teagasc, Johnstown Castle, Co. Wexford. Pig, cow and chicken manure was applied to grassland. Manure samples were taken before application to the plots and samples of soil and grass were taken over a period of 4 months. Samples were cultured on EMB agar and Simmons Citrate agar supplemented with cefotaxime (4mg/L), colistin (4mg/L), ciprofloxacin (1mg/L) and kanamycin (64mg/L) to select for *E. coli* and *K. pneumoniae*, respectively. Isolate identities were confirmed by MALDI-TOF.

Results: In total 64 *E.coli* isolates were isolated; 7 isolates from pig manure, 14 from cow manure, 0 from poultry manure, 11 from soil and 14 isolates from grass. Seven *K. pneumoniae* isolates were identified, all of which were from grass samples. Isolates underwent disk diffusion testing according to EUCAST (2019) guidelines. Of the *E. coli* isolates 36 were resistant to tetracycline, 9 resistant to cefotaxime, 24 resistant to kanamycin and 12 resistant to ciprofloxacin. Two *K. pneumoniae* isolates were resistant to amikacin.

Conclusions: The results from this study show that soil, and notably, the grass phyllosphere, can act as a resevoir of antibiotic resistant bacteria of public health concern.

[P157] IMPORTED HYALOMMA TICKS IN THE NETHERLANDS 2018-2020

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Aim: Ticks of the genus *Hyalomma*, which are vectors for several tick-borne diseases, are occasionally found in areas outside their endemic range including northern parts of Europe. The objective of this study was to analyse adult *Hyalomma* ticks that were recently found in The Netherlands.

Methods: *Hyalomma* ticks were morphologically identified. Cluster analysis based upon sequence data (*cox1* barcoding) for molecular identification and pathogen detection was performed. Additionally, a cross-sectional survey among horses was conducted to actively search for *Hyalomma* ticks in summer 2019. Analysis of temperature was done to assess the possibility of i) introduced engorged nymphs moulting to adults and ii) establishment of populations in The Netherlands.

Results: Seventeen adult *Hyalomma* ticks (one in 2018, eleven in 2019, five in 2020) were found by citizens and reported. Fifteen ticks were detected on horses and two on humans. Twelve were identified as *H. marginatum*, one as *H. rufipes* and four, of which only photographic images were available, as *Hyalomma* sp. No Crimean-Congo Hemorrhagic Fever virus or *Babesia/Theileria* parasites were detected. One adult tick tested positive for *Rickettsia aeschlimannii*. In the cross-sectional horse survey, no *Hyalomma* ticks were found. Analysis of temperatures showed that engorged nymphs arriving on migratory birds in spring were able to moult to adults in 2019 and 2020, and that cumulative daily temperatures in The Netherlands were lower than in areas with established *H. marginatum* populations.

Conclusions: Our results show that *Hyalomma* ticks are regularly introduced in The Netherlands as nymphs and these are able to develop to the adult stage under the Dutch weather conditions. Establishment of permanent *Hyalomma* populations is considered not likely. Vigilant citizens can notify *Hyalomma* adult ticks, especially when attached to horses. Only one human pathogen, *Rickettsia aeschlimannii*, was found in one of the ticks. The risk of introduction of tick-borne diseases via *Hyalomma* ticks on migratory birds is considered to be low.

[P158] EVALUATION OF THE CONTAMINATION OF LETTUCES BY ECHINOCOCCUS MULTILOCULARIS EGGS

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Aim: Human alveolar echinococcosis (AE) infection is caused by oral ingestion of microscopic parasite eggs. Despite very few and heterogeneous data are available on the exact route of infection to humans, foodborne transmission is considered as one of the most important. This study aims to produce data on the contamination of lettuce by *Echinococcus* and other *Taenidae* species.

Methods: One hundred and six lettuces, corresponding to 35 pools of 300 g from two to four lettuces were sampled from private kitchen gardens or local markets from two French high endemic region for *E. multilocularis* (*Em*).

Results: The limit of detection for *Em* was established at three eggs in 300 g of lettuces. Two pools of lettuce were positive for *Em* both from local markets, while six others were contaminated by *Hydatigera* sp. mainly from private kitchen gardens.

Conclusions: Even if the viability of the eggs can't be proved, the detection of *Em* suggests that lettuce can be a source of infectionas also highlighted by the detection of *Hydatigera* sp. A European multicenter study will be conducted in 2021 inside Meme project to obtain data from different epidemiological situations.

Funding: This reasearch within MEME project is supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

[P159] EDUCATING THE GENERAL PUBLIC ABOUT INFECTIOUS DISEASES UTILIZING A SOCIAL PLATFORM: EXPERIENCE FROM A YEAR-LONG PILOT

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Aim: Infectious diseases are an important public health problem, and the general public plays a pivotal role in minimizing its spread. The dissemination of scientific information in an understandable manner is one of the strategies to expand the knowledge and awareness among society. We have launched and maintained a social media platform through the scope of one year to meet this objective.

Methods: A total of 193 contents were created between June 2020 and February 2021. A wide variety of public health topics have been covered, primarily zoonoses, antimicrobial resistance, climate change, epidemiology and history of medicine. A special focus was put on facts and myths about SARS-CoV-2 related topics. Primary and secondary literature reviews were performed to have fact-checked information. Multiple social media channels were utilized for the dissemination of contents. Knowledge assessment was performed utilizing knowledge survey questions.

Results: This pilot initiative of this platform has acquired more than 2100 followers on its Instagram, Facebook and Twitter pages. Most followers were living in the United States (30.8%), followed by India (19.9%) and Canada (4.6%). The highest engagement was seen for pandemic-related content, followed by climate change and bacteriology. The age of the online community ranged between 13 to 65 years, and the majority was female (60.0%). The reach increased weekly by approximately 2%.

Conclusions: The pilot phase has led to a high reach within the general public, meeting the aim of this project. These observations highlight the necessity of a digital platform that promotes infectious diseases among the general public.

References:

https://www.instagram.com/infectiousdiseasedaily/

https://www.facebook.com/infectiousdiseasedaily/

https://twitter.com/infectiousdaily

[P160] ESTABLISHING EPIDEMIOLOGICAL CUT-OFF VALUES (ECOFFS) FOR STAPHYLOCOCCUS PSEUDINTERMEDIUS AND S. HYICUS

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Aim: Setting of epidemiological cut-off values (ECOFFs) supports the process of defining animal-specific clinical breakpoints for veterinary antimicrobials and improves harmonization in the monitoring of resistance in animal pathogens. This project aimed to establish missing ECOFFs for antimicrobials for veterinary use by performing susceptibility testing of animal bacterial pathogens.

Methods: Within OHEJP IMPART antimicrobial susceptibility testing (AST) of animal bacterial pathogens was performed by determining the Minimum Inhibitory Concentration (MIC) of twenty-six different antimicrobials with broth microdilution according to international guidelines (ISO, CLSI) using customized plates (Sensititre©). MIC data were curated by following EUCAST Standard Operating Procedure (SOP) 10.1. Next, ECOFFs were calculated using the ECOFFinder programme (network version 2.1).

Results: Nine partner institutes performed AST on 2,831 bacterial isolates involving nineteen different animal bacterial pathogens. This resulted in 1,310 MIC-distributions consisting 47,640 MIC-values of 34 different antimicrobials. As a first result, eight tentative ECOFFs were calculated for *Staphylococcus pseudintermedius*, including cephalexin, clindamycin, cloxacillin, enrofloxacin, florfenicol, gentamicin, lincomycin, and neomycin. For *Staphylococcus hyicus*, five tentative ECOFFs were set, including cephalexin, doxycycline, enrofloxacin, tetracycline, and trimethoprim/sulfamethoxazole.

Conclusions: After formal approval by EUCAST, the calculated ECOFFs for *S. pseudintermedius* and *S. hyicus* will become publicly available for veterinary diagnostic and research laboratories. The large set of MIC data produced within OHEJP IMPART will boost the setting of ECOFF for other animal bacterial pathogens. Subsequently, PK/PD and clinical outcome data will be needed to define animal-specific clinical breakpoints.

Funding: This project is supported by the One Health EJP

[P161] IMMUNE RESPONSE, GUT MICROBIAL COMPOSITION, SALMONELLA SUPER AND LOW SHEDDER PHENOTYPES CAN BE MODULATED BY THE INOCULATION OF FOUR COMMENSAL BACTERIA IN CHICKEN.

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Aim: Many studies have revealed the importance of heterogeneous shedding levels in the context of infectious diseases. Individual hosts that excrete higher levels of pathogens (so-called super-shedders) are key targets for control strategies. However, the mechanisms associated with the emergence of super-shedders remain largely unknown.

The development of a new infection model for chickens reared in isolator, where animal reinfections are greatly reduced, has allowed us to demonstrate that two main *Salmonella* Enteritidis shedding phenotypes can emerge within the same chicken genetic background. These phenotypes were designated as super- and low-shedder on the levels of *Salmonella* faecal excretion and caecal colonization.

Methods and results: In this project, we analysed the parameters that could be responsible for these phenotypes, namely: 1- the modification of the virulence of the *Salmonella* strain during the asymptomatic carrier state ; 2- the faecal and caecal microbiota composition ; 3- the immune status of chicks measured in kinetic in blood. We also analysed the impact on these parameters of four commensal bacteria inoculated before infection.

Conclusions: The data obtained showed the protective effect of the four commensal bacteria on *Salmonella* excretion and their major impact on gut microbiota composition and immune response.

Funding: The study is funded by the EJP via the MoMIR-PPC project.

[P162] WHOLE GENOME SEQUENCING (WGS) BASED SURVEILLANCE OF PEDIATRIC HEMOLYTIC UREMIC SYNDROME (HUS) CAUSED BY SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) IN ITALY, 2016-2020.

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[2] Istituto Superiore DI Sanità

Aim: Hemolytic Uremic Syndrome is the leading cause of acute renal failure in children. In Italy, surveillance of HUS aims to monitor the epidemiology of STEC infections and to early detect outbreaks. Here we summarise the results of the surveillance between 2016 and 2020.

Methods: Stool and blood samples from HUS patients referring to nephrology units, are tested for STEC, by Real-Time PCR for the detection of *stx* genes, classical microbiology and serology. STEC isolates are characterised by WGS to refine the characterization through serotyping, MLST, virulotyping and to perform cluster analysis by cgMLST.

Results: 322 HUS cases were identified corresponding to 0.72 mean annual cases per 100,000. Top-5 STEC serogroups (O26, O111, O157, O145, O103) were identified in 214 cases and prevailed among the 247 STEC positive HUS cases. STEC O80 was diagnosed in 9 cases compared with just a single case in the previous period. A total of 140 STEC isolates from patients or their family members were characterised by WGS. The comparative genomic analysis allowed to identify 14 clusters (< 15 alleles difference). Nine clusters included strains from patients with no clear epidemiological link.

Conclusions: Although in 2020 the HUS reporting dropped remarkably during the COVID-19 lockdown, cases increased of 22% compared with the six previous years. The use of the WGS allowed the identification of rare STEC serogroups. The identification of isolates with a high genetic similarity in different years suggests that certain STEC clones may circulate repeatedly over time leading to persistent contamination of the sources.

[P163] THE PESENCE OF ESBL-PRODUCING E. COLI AMONG COWS AND CALVES ON AUSTRIAN DAIRY FARMS

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Aim: The aim of the present study is to investigate the presence of Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* on dairy farms in Austria.

Methods: In summer 2020, faecal samples were collected from 51 farms distributed throughout Austria. These farms kept primarily dual-purpose *Fleckvieh* cows with an average herd size of 43 head.

Dairy cows were sampled with two pairs of boot swabs per farm. Samples were taken from the alleys. Weaned calves (>6 weeks; max. 5) and pre-weaned calves (<6 weeks; max. 5) were sampled using rectal swabs. One pair of each boot swabs and rectal swabs per age group were pooled in the laboratory. Samples were analysed and evaluated according to an adapted EURL-AR ESBL detection method within 96h of sample collection.

Results: A total of 204 samples were examined and 202 could be evaluated.

On 84.3% of the farms at least one faecal sample was positive for ESBL-producing *E. coli*. Of the 51 farms, 18 (35%) contained ESBL-producing *E. coli* in all three production groups and only 8 farms (15.7%) had a negative result in all samples.

Overall, 76% of pre-weaned calf pools, 66% of weaned calf pools and 58% of dairy cow samples tested positive for ESBL-producing *E. coli*.

Conclusions: Further studies are needed to identify the risk factors which contribute to the high prevalence in cows and calves and the development and transmission of the bacteria. Furthermore, the risk for humans, animals and the environment posed by ESBL-producing *E. coli* on farms needs to be analysed.

[P164] THE DEVELOPMENT OF AN AGE STRUCTURED MODEL TO DESCRIBE THE TRANSMISSION DYNAMICS OF CYSTIC ECHINOCOCCOSIS IN ARGENTINA.

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Aim: Mathematical models are an important tool for the quantification of infectious diseases and can be used to predict disease spread, as well as evaluate different disease control measures. Here, we introduce an age stratified multihost model for the simulation of Cystic echinococcosis in Argentina.

Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by infection with the cestode species *Echinococcus granulosus sensu lato* and is a globally distributed neglected tropical disease (NTD). It particularly affects vulnerable sheep-rearing and pastoral communities, with a significant economic burden to both the health and agricultural sectors. Elimination has been achieved in other countries such as New Zealand and Iceland. Control programmes use a combination of dog deworming, less frequently sheep deworming, and annual sheep vaccination, with large reductions in prevalence achieved in the last decades.

Methods: The model simulates transmission dynamics in a population of dogs and sheep within a single farming unit, representative of the style of pastoral sheep farming (predominantly for wool) seen in the region.

Results: Model outputs will be used to provide insights into the epidemiology of CE in Argentina and will allow for a rigorous evaluation of current control efforts.

Conclusions: Alternative intervention scenarios will be modelled, to identify the most effective and economically viable strategy for the long-term sustainability of the programme.

[P165] CROSS SECTORAL QUALITY ASSURANCE SYSTEMS, AN INVENTORY

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Aim: Task 1 of Workpackage 1 of the EJP-CARE project concerned an inventory on existing quality assurance (QA) schemes, being Proficiency Tests (PTs) or External Quality Assurance schemes (EQAs), for characterizing (bacterial) food borne pathogens. The aim was to investigate the availability of QA schemes for molecular methods, preferably WGS/NGS based methods, that can be used cross-sectoral within the OneHealth framework.

Methods: For the inventory several sources of information were used:
1. a website[1] with information on different QA-schemes for a wide range of applications. The seärched testing field was ”Microbiology”. Selections were based on descriptions of the PTs/EQAs.
2. information on QA-schemes for molecular methods obtained from several European Union Reference laboratories (EURLs) and ECDC.

Results: Most QA-schemes on the indicated website deal with isolation/detection and phenotypic characterization of food borne microorganisms. In some cases molecular methods may be applied for species characterization. Several EURLs organize PTs for characterization and/or cluster analysis of bacterial pathogens, but these PTs are only intended for the National Reference Laboratories in the food/veterinary sector. Additionally, similar QA schemes have been organized by ECDC, but these schemes are only intended for the National Reference Centres in the human sector.

Conclusions: Currently, no cross sectoral PTs/EQAs are organized for characterization and/or cluster analysis of foodborne pathogens using WGS/NGS. To gain information on the uniform quality of analysis in both sectors during foodborne outbreaks, it is recommended to set up these type of PTs/EQAs for cross sectoral evaluation of bacterial strains.

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[P166] SALMONELLA PREVALENCE AND SEROVAR DISTRIBUTION IN DUCK FLOCKS

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Aim: *Salmonella* is considered one of the major zoonotic factor affecting humans and carring mainly in poultry and poultry products. EU coordinated control programmes for *Salmonella* targeting selected poultry species like chickens and turkeys. Little data are available on the rate of *Salmonella* occurrence in ducks. The aim of the study was to assess prevalence and antimicrobial resistance of *Salmonella* in duck flocks.

Methods: In 2019**,** 304 boot swab samples taken from 152 duck flocks (two samples deriving from different area per flock) were tested for Salmonella according to PN-EN ISO 6579 PN-EN ISO 6579:2002/A1:2007 standard. Isolates were identified according to White–Kauffmann–Le Minor scheme using slide agglutination tests with commercial antisera. Antimicrobial susceptibility testing (AST) of confirmed *Salmonella* isolates was performed with broth microdilution method (Sensititre EUVSEC plates; TREK Diagnostic Systems). Epidemiological cut-off values (ECOFFs) according to the EUCAST were used as interpretation criteria for minimal inhibitory concentration (MICs) obtained for 14 compounds representing 9 antimicrobial classes (beta-lactams, quinolones, phenicols, folate-path inhibitors, tetracyclines, polymyxins, macrolides, and glycylcyclines). For each substance with MIC above the cut-off, the isolate was regarded as microbiologically resistant (non-wild type, NWT). Duplicate strains deriving from the same farms were excluded.

Results: *Salmonella* was confirmed in 58,6% (175/304) of tested samples and in 61,2% (93/152) farms. In 23,6% (22/93) of tested flocks two different serovars per farm were identified. Overall, 175 *Salmonella* isolates belonging to 12 different serovars and serological forms were obtained. The most prevalent were: *S*. Enteritidis (n=35), *S*. Senftenberg (n=29), *S*. Anatum (n=23), *S*. Indiana (n=18), *S*. Infantis (n=18), *S*. London (n=9), and *S*. Newport (n=9). Seventheen strains showed autoagglutination. The overall level of microbiological resistance among tested *Salmonella* remained low except to chinolones, where 40,2% (45/112) isolates were resistant to ciprofloxacin and 24,1% to nalidixic acid. 11,6% of isolates were resistant to ampicilin. All of 58 (51,7%) *Salmonella* were pan-susceptible. One of the two AST tested isolates of *S*. Give was identified as multidrugresistant (AmpCipNalColSulTmp).

Conclusions: The study have shown that ducks are serious reservoir of diverse *Salmonella* serovars. Although ducks are resilient to systemic infection caused by *Salmonella*, they may shed it in the feces. It might pose a risk of infection for the farm workers and consumers of ducks products. Longitudual investigation is neccesarry to assess reall overall *Salmonella* prevalence and persistance in duck flocks with major focus on *S*. Enteritidis.

Funding: Laboratory study was performed within task 16 of Multiannual Research Project (2019 – 2023). Current analysis was partially supported by Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards - One Health EJP GA No 773830.

[P167] GIS AS A TOOL FOR MAPPING SALMONELLA SEROVARS IN PIGS IN POLAND BETWEEN 2014-2018

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Aim: The aim of the study was to use Geographical Information System (GIS) for mapping occurrence of Salmonella serovars in pigs within country-wide monitoring in Poland between 2014-2018.

Methods: The input data accounted for number of pigs at the level of counties, were obtained from General Veterinary Inspectorate and Salmonella tests results were obtained from National Reference Laboratory for Salmonella and AMR (Dept. of Microbiology, PIWet). The localisations of top-five serovars: S. Derby, S. Enteritidis, S. Infantis, S. Typhimurium, monophasic S. Typhimurium, and other Salmonella serovars were presented on maps generated using ArcGIS 10.4.1 (ESRI).

Results: The maping showed that Salmonella serovars localisations were centrered in the regions of Poland with higher pig density. In some areas monophasic S. Typhimurium and S. Derby occured every year. S. Enteritidis was observed only in a few localisations each year. In 2014, all presented localisations of Salmonella serovars were spread all over the country. In 2015 less Salmonella localisations were observed in Eastern Poland compared to 2014. In 2016 and 2018 a single localisations of S. Infantis were noted in the centre of the country. Moreover, only in 2017 several Salmonella serovars including Infantis and Typhimurium were observed in one county in Western Poland.

Conclusions: Results of Salmonella serovars mapping might be useful tool for stakeholders and scientists for spatio-temporal analysis allowing for designation of problematic areas.

Funding: Salmonella study was performed within task 12 of Multiannual Research Project (2014 – 2018). Current analyses were done in One Health perspective. Research was partially supported by Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards. - One Health EJP GA No 773830.

The logo is shown under abstracts that the authors indicated to be from One Health EJP activities.

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